

# **The Impact of Trypanosomiasis on Ethiopian Livestock**

**by**

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A thesis submitted for the degree of Master of Veterinary Medicine in the Faculty of  
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## **Declaration**

I hereby declare that this thesis is entirely my own work and confirm that it has not been submitted to any University for the award of a degree.

## **Abbreviations**

AACM	Australian Consulting and Management
AAU	Addis Ababa University
ADB	African Development Bank
AI	Artificial Insemination
AISCO	Agricultural Inputs Supply Corporation
CBPP	Contagious Bovine Pleuro Pneumonia
CCPP	Contagious Caprine Pleuro Pneumonia
CFT	Complement Fixation Test
DDE	Dairy Development Enterprise
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agricultural Organisation
FLDP	Fourth Livestock Development Project
FMD	Foot and Mouth Disease
GDP	Gross Domestic Product
IAR	Institute of Agricultural Research
ILCA	International Livestock Centre for Africa
ILRAD	International Laboratory for Research on Animal Disease
MOA	Ministry of Agriculture
MSFD	Ministry of State Farms Development
NAE	National Atlas of Ethiopia
NCS	National Conservation Strategy
NGO	Non Governmental Organisations
NTTICC	National Tsetse and Trypanosomiasis Investigation and Control Centre
ONCCP	Office of the National Committee for Central Planning
PARC	Pan African Rinderpest Campaign
TCP	Technical Co-operation Project
TLDP	Third Livestock Development Project
TLU	Tropical Livestock Unit
WB	World Bank

## Summary

This study examines the impact of trypanosomiasis on the livestock industry of Ethiopia and discusses the need for further research, diagnosis and control in the future.

The thesis begins with a brief introduction to the country's geographical location and its livestock industry. The institutions involved in research and extension to improve the livestock sector and supported by the government and non-governmental organisations are described.

The first chapter is a review of the epidemiology of trypanosomiasis in Ethiopia (past and present). This includes the distribution of tsetse-flies, their progress to higher altitudes, the ecological factors involved in the tsetse advance and the role of tsetse as vectors of trypanosomes. Tsetse and non-tsetse transmitted trypanosomiasis and their occurrence in relation to the tsetse distribution and the prevalence of trypanosomiasis in domestic livestock and humans are discussed.

The main emphasis of the third chapter is an assessment of the socio-economic impact of trypanosomiasis in Ethiopia. This involves livestock production systems, the importance of livestock in the Ethiopian economy and the interaction between livestock and crop production particularly in the highlands and investigates the reasons why livestock production is precluded from most of the lowland areas of the country. In order to quantify the direct economic losses due to trypanosomiasis in the tsetse-infested areas of Ethiopia assumptions are made on the number of livestock at risk, the prevalence of the disease, mortality rate for different species of domestic livestock and the prices of livestock and livestock products based on previous studies and current prices in Ethiopia and neighbouring countries. The direct production losses, through mortality, morbidity and infertility account for about 97% of the total and research, investigation and control costs incurred by the National Tsetse and

Trypanosomiasis Investigation and Control Centre (NTTICC) accounts for 3% of the total direct cost. It is recognised that indirect losses caused by the disease in preventing or impairing agricultural development are far greater than the direct losses. However indirect losses are very difficult to quantify because the development of the tsetse-infested areas requires an integrated lowland development scheme. This chapter takes account of the seriousness of the problem and assesses different control methods and future control options.

The third chapter provides a review of *Trypanosoma equiperdum* in equines. The historical background of the disease, its distribution, morphology, transmission, clinical signs, pathogenesis, diagnosis, immunity and control measures are discussed.

The fourth chapter presents results of a survey of horses in the Arsi and Bale regions of Ethiopia using ELISAs to detect antibodies to, and antigens of *T. equiperdum*. The study was focused on seven selected sites and 309 horses of both sexes were clinically examined and blood sampled. Testing of the sera was initially carried out in Ethiopia. Later all 309 sera were retested in Glasgow to confirm the results. Blood and genital washes from seven antigenaemic horses (5 female and 2 male) were inoculated into laboratory rodents in an attempt to isolate the parasites but none developed a patent parasitaemia. The ELISA results showed that 101 horses had high antibody positivity, whilst only 19 horses gave high antigen positivity when compared with strong positive controls. The results obtained from the antibody and antigen ELISAs were analysed and correlated with the clinical signs ( $p < 0.001$  and  $p < 0.01$ ) respectively.

The study provided clear evidence that *T. equiperdum*, which is the causative agent of dourine in equines, occurs in the Arsi and Bale regions of Ethiopia. All the three investigation methods (clinical observation, serology and laboratory animal inoculation) provided valuable evidence towards this conclusion. All the study sites were in the highlands, and at altitudes outside the known tsetse-infested areas and there was no evidence of *T. evansi* infections in

the areas of study. The significant correlation of clinical signs observed in the field with antibody positive and antigen positive horses gives further support for the existence of dourine in the study areas. Since equines travel long distances freely for trade and transport purposes in Ethiopia it is believed that the disease may have a much wider distribution than the study areas and especially in places where there are large equine populations.

A number of recommendations are made for further studies to determine the incidence and distribution of *T. equiperdum* amongst the equine population of Ethiopia and the development of appropriate control strategies.

## Introduction

Ethiopia is an agrarian country with an estimated population of 47 million people. The population is growing at an annual rate of 2.9% and is predominantly rural (Goshu *et al.*, 1989). The country occupies the interior of the Eastern horn of Africa extending along a thousand kilometre long coastline of the Western shore of the Red Sea. In size, Ethiopia has an area of 1,223,000 square kilometres stretching 30° north of the equator to latitude 18° north of the equator and from 33° east to 48° east longitude (NAE, 1988). Agriculture which is the dominant sector of the economy accounts for over 50% of the GDP and 35% of the export revenue, and provides a livelihood for over 80% of its inhabitants (Goshu *et al.*, 1989). It is widely recognised that varied agro-ecological conditions render Ethiopia a suitable country for many breeds or types of livestock, it is, therefore, no accident that the livestock population of the country accounts for about 17% of the cattle, 15% of the sheep and goats, 49% of the equine population in Africa. In addition, there are considerable wild game, marine life and apicultural resources (Mullea, 1989).

There has never been a detailed identification or classification of Ethiopian cattle breeds and varieties. Broadly there are two types: Senga and Zebu. Within the Senga type there are the Danakil/Afar/Kereyu, Horo and Fogera breeds. The Zebu type includes such breeds as Barka, Borena and Arsi (Kebede *et al.*, 1988).

There are three major production system zones the cereal/livestock (highlands) system, the pastoral (dry lowlands) system, and the perennial/livestock (humid) production system, the differences being due to the ecology of the particular area. The interaction between ecology and livestock also introduces a distinct set of animal environments influenced by climate stress, feed, water supply, and disease hazard (Goshu *et al.*, 1989).

Despite the country's potential, which is enormous by sub-Saharan African standards, the contribution of livestock to the agricultural GDP is considerably below that which could reasonably be expected - by about 30% (Mullea, 1989) and it is



projected to fall further to 26% by the year 2000 (FAO as cited by Goshu et al., 1989). The major proportion of the livestock resource (74%) is owned by cultivators and the remainder (26%) by pastoralists. Production from cattle has been estimated to be 620,000 tons of milk, 244,000 tons of meat, 24 million tons of manure and 2.4 million hides annually. The per capita consumption of milk is estimated to be 19 litres a year, while meat consumption is 13.9kg a year which beef and veal contribute 6.4 kg and sheep, goats, chicken and camels the rest (National Conservation Strategy 1991). There have been several reasons given to explain the poor performance of the livestock sector. The major constraints to livestock production are similar to those of other countries of the region in sub-Saharan Africa. These constraints can be broadly categorised as technical, socio-economic and institutional. The technical constraints include shortages of feed and nutrition, genetic factors and the widespread prevalence of disease. Socio-economic and institutional constraints include insufficient capital investment and recurrent expenditure, lack of appropriate technical packages, underdeveloped infrastructure, lack of qualified and experienced personnel, illegal livestock movements across the borders and lack of development policy (Negussie, 1988).

However, geographically, Ethiopia is ideally placed to export animals and animal products to the enormous markets of the Middle East and the smaller and still substantial markets of North and West Africa. In 1985, these markets absorbed nearly 10 million sheep and goats and 0.2 million cattle. Of the total market worth Birr 1.6 billion, Ethiopia's share was under 1%. During the same year the country exported 624 tonnes of chilled and frozen beef and mutton with a total value of Birr 1.9 million. The dominant markets were the Yemen Arab Republic and Saudi Arabia. Of an annual off-take of 2.0 - 2.6 million head of cattle, 92% is consumed locally and only 8% is exported (Negussie, 1987).

It is known that the growth of the livestock industry depends on research, extension and implementation, and these activities in Ethiopia are still at an infant stage. Government efforts have been made to improve this subsector by establishing

governmental institutions responsible for livestock development that can assess and formulate guidelines for identification and classification of the country's livestock wealth for breeding, import, export and distribution of stock. Institutions engaged in activities in the livestock subsector are the Institute of Agricultural Research (IAR), Ministry of Agriculture (MOA), Ministry of State Farm Development (MSFD) and institutions of higher learning such as Alemaya University (AU). IAR is responsible for generating, adopting, and diffusing technologies and fostering scientific activities through national and international conferences, workshops and seminars relevant to the livestock subsector. Higher learning institutions are mandated to produce a trained workforce for the subsector and to conduct research that will facilitate the teaching-learning process. This will also contribute to the development of the subsector. MOA, apart from initiating policy guidelines for the country's agriculture sector and preparing priority strategies, is largely responsible for overseeing the development of the subsector particularly among the peasantry. For this purpose MOA is organised into four main sections out of which Animal and Fisheries Resources Development is the main department and is responsible for livestock development activities. The principal areas of operations are animal production and nutrition, animal health services, livestock marketing and rangeland development.

### **Livestock production and nutrition**

The Ethiopian livestock population consists of indigenous stock which is characterised by very low productivity. The capacity to produce more milk, meat, wool/hair, and draft power could be increased by selection, cross breeding or by importing exotic breeds. However, as the potential for productivity increases, the resistance to disease and the tolerance of adverse conditions decreases, thus demonstrating that genetic improvement is a complex problem which influences livestock development in the country.

However, based on the emphasis given to the subsector, livestock improvements in production and nutrition are underway to improve the productivity of indigenous breeds by the distribution of improved stock, the operation of

multiplication centres and ranches, the provision of artificial insemination (AI) services, the introduction and promotion of proper grazing management and the production of cultivated forages. These services are operational through: the Abernossa cattle ranch, Gobe cattle ranch, Bege cattle ranch, Metekel cattle ranch, Debre Berhan sheep ranch, Amed Guya sheep ranch, Kaliti AI centre, Asela AI centre and Asmara AI centre. Exotic cattle have also been introduced to selected highland areas to upgrade the productivity of Ethiopian livestock. Some of these exotic breeds are Friesian, Jersey and Simmental.

The major feed resources in Ethiopia include natural pasture grazing / browsing, crop residues and agro-industrial byproducts and to a lesser extent improved pasture and forage crops. The amount and quality of native pastures available to livestock vary with altitudes, rainfall, soil type and cropping intensity. The total area available for grazing and browsing is 62 million hectares, of which 12% is in farming areas and the rest is distributed over very large pastoral areas. The scope for decisive improvements both in quantity and quality of native pastures in the highlands seems very limited, because natural grassland in the Ethiopian highlands is generally confined to degraded, shallow upland soils which cannot be successfully cropped because of physical constraints such as flooding and water logging. The native pasture land in the Ethiopian highlands provide not more than half of all animal feed, the remainder being crop residues. However, the new initiatives under FLDP and other research institutions in forage development look promising and the farmers' response indicates intensification of forage production and feeding systems can provide the basis for a shift to much more control of livestock grazing and cut-and-carry management.

### **Livestock marketing**

One of the factors responsible for the low contribution of the subsector is the underdevelopment and disorganisation of the country's livestock marketing system. The system is characterised by a large number of widely dispersed markets with no facilities. Only recently the Second Livestock Development project, MOA and FAO-

TCP-projects introduced essential facilities such as perimeter fencing, pens, weighing scales, crushes, water and watering troughs, offices and latrines in a limited number of markets. These recent efforts were directed towards the introduction and improvement of the marketing of animals on the basis of weight and body condition, establishment of modern slaughterhouses, hide and skin stores and stock routes, along which fenced grazing reserves or staging points were constructed.

### **Animal health**

The Veterinary Services Department of the MOA is responsible for the protection of animal health in Ethiopia. Although the eradication of diseases of economic importance from the country still has a long way to go significant advances in reducing the mortality rate caused by diseases such as rinderpest, foot-and-mouth disease (FMD), and contagious bovine pleuropneumonia (CBPP), anthrax and other diseases have been achieved by vaccination. Widespread epidemics no longer occur, but sporadic outbreaks of these diseases still occur and their presence adversely affects the country's international trade in animals and animal products. In the lowland area of the country the major animal health problem is undoubtedly tsetse-transmitted trypanosomiasis.

Short and long-term health programmes emphasise veterinary epidemiology, control of diseases and their vectors, clinical treatments and vaccination, and the production of vaccines and other biologicals supported by manpower and infrastructural development.

Studies and investigations falling under the short-term programme with special regard to trypanosomiasis and tsetse, rinderpest, CBPP, FMD, contagious caprine pleuropneumonia (CCPP), rabies, tuberculosis, brucellosis and tick-borne disease are those carried out by the National Tsetse and Trypanosomiasis Investigation and Control Centre (NTTICC), the National Veterinary Institute(NVI), the existing five regional laboratories and the planned Central Disease Investigation Laboratory;

## **Rangeland improvement**

The rangelands of Ethiopia cover about 60% of the total land mass. About 3 million pastoralists inhabit this area with 20% of the cattle, 25% of the sheep, 75% of the goats and all the camels in the country. Although the rangelands account for less than one-third of the livestock population in the country, their potential contribution to export earning is high. Currently, nearly all live animals exported and most animals supplied to industrial abattoirs originate in the lowlands where they are produced under traditional management systems. Traditional pastoral systems favour livestock numbers as opposed to production per head.

Recently research and development activities have been initiated through the collaborative efforts of the Third Livestock Development Project and other organisations to introduce an integrated pilot range management system. The project has several components of which range land and water development, veterinary and animal health programmes and co-operative ranch management are the most promising.

Apart from the recurrent and capital budgets allocated to these programmes there are also other major livestock projects for which financing has already been secured from a number of agencies. Most of these projects are financed by bilateral aid, non-governmental sources and the Central Treasury of Ethiopia, eg. the Third and Fourth Livestock Development Projects, the Pan African Rinderpest Campaign (PARC).

The livestock development programme in the MSFD is under the Ethiopian Livestock and Meat Corporation established with the objective to expand and manage commercially oriented livestock development enterprises and related agro-industries, wherever feasible throughout the country in order to contribute towards the satisfaction of local demand with respect to its products, earn as much foreign currency as possible by competing in international markets and save foreign currency by reducing the importation of livestock products.

Programme activities include : managing dairy, poultry and pig farms; managing meat, dairy and animal feed processing plants; processing, finishing (cattle only) and exporting live animals (mainly ruminants); marketing products in local and foreign markets; planning, executing and expanding new livestock development projects.

However the problem of disease has exerted a profound influence on livestock distribution and productivity in this country. The prime example is trypanosomiasis which seriously affects the livestock industry.

This thesis is concerned with the impact that trypanosomiasis has on livestock development in Ethiopia.

## Section I

### Chapter I

#### Epidemiology of Trypanosomiasis in Ethiopia

Trypanosomiasis is a parasitic disease caused by species of flagellate protozoa belonging to the genus *Trypanosoma* which inhabit the blood plasma and various body tissues and fluids. These parasites are found in many animals but seem to be pathogenic only for mammals, including man (Finelle, 1983). In sub-Saharan Africa, the disease is spread mainly by the bite of trypanosome-infected tsetse-flies (genus *Glossina*). Trypanosomiasis is considered the most important infectious disease holding back the development of livestock in most parts of Africa (ILRAD, 1993). The disease has been recognised for many years (Bruce, 1895; Buxton, 1955; Nash, 1960, 1969; ), yet it remains endemic in the animal and human populations across sub-Saharan Africa. The magnitude of the problem forced the World Food Conference in 1974 to call upon FAO to launch as a matter of urgency, a long term programme for the control of African animal trypanosomiasis. Accordingly, in 1975 FAO initiated a programme for the control of African trypanosomiasis and related area development to which very high priority was given (FAO, 1983). However, the problem is still prevalent in 37 African countries and over 11 million km<sup>2</sup> (one third of the continent) area is infested by tsetse (Jordan, 1986).

The occurrence and spread of trypanosomiasis in African livestock are determined primarily by the degree of contact between the domestic animal and *Glossina*, while transmission by other bloodsucking *Diptera* is usually of secondary importance. In turn, the role of tsetse-flies as vectors of 'nagana' is closely related to the source of their food, represented chiefly by wild ungulates, a significant proportion of which are symptomless carriers of trypanosome that are pathogenic to domestic stock. The infection circulates among wild game animals independently of domestic animals. These become involved when exposed to attacks by tsetse-flies in

natural enzootic foci, but once the disease establishes itself in domestic animals they too become sources from which it spreads further among livestock (Hoare, 1972).

The earliest history of trypanosomiasis in Ethiopia is in accounts given by explorers and travellers telling of losses of their transport animals when they had encountered tsetse-fly belts. One of the earliest records was made by Donaldson Smith in 1885, who in his accounts of his journey from Somalia through southern Ethiopia to Lake Rudolph mentions that the Gendi-fly (tsetse) attacking his transport animals caused a serious disease called Gendi (trypanosomiasis) from which many animals died (Langridge, 1976). According to Krug (1971) a 1956 FAO report mentioned the occurrence of trypanosomiasis in Shoa and Eritrea provinces only. No further information appeared until 1961 when two FAO reports mentioned that the disease was already recognised in the west of the country bordering the Sudan, where the tsetse-fly belts appeared to be extending. The native population believed that the infecting agent was carried by migrating buffaloes. The occurrence of tsetse-borne trypanosomiasis has caused the recent abandonment of good arable land in some areas. The disease occurs in the following regions and provinces: Maji, Kaffa, Gamu Gofa and Wellega, extending along the valleys of the Omo and Ghibe (Upper Omo) rivers. The reports stressed the necessity for close study of the tsetse-fly by specialised personnel and the increasing danger of drug resistance due to under-dosing and haphazard use of drugs. In 1967 FAO reported that the boundaries of trypanosomiasis incidence were still uncertain but seemed to be far more widespread than had originally been thought.

As stated by Bergeon and Ballis (1969) the economic aspect of livestock loss and disruption to the people was extremely serious, but urgency was added to the problem when in 1967 doctors at the American NAMRU III tropical medicine research unit in Addis Ababa reported seven cases of human trypanosomiasis -one at Bako and six at Gambella in Illubabor. During this period great efforts were made by the Italians under Siero Vaccinageno in Asmara, the French under the Mission



Veterinaire Francaise en Ethiopie and the British under the Ministry of Overseas Development and they carried out substantial work on trypanosomiasis in Ethiopia. Since 1977 this work has been continued by the Ethiopian Trypanosomiasis Control Services (TCS), of the Ministry of Agriculture which later become the National Tsetse and Trypanosomiasis Investigation and Control Centre (NTTICC, 1986).

### **The Current Trypanosomiasis Situation in Ethiopia**

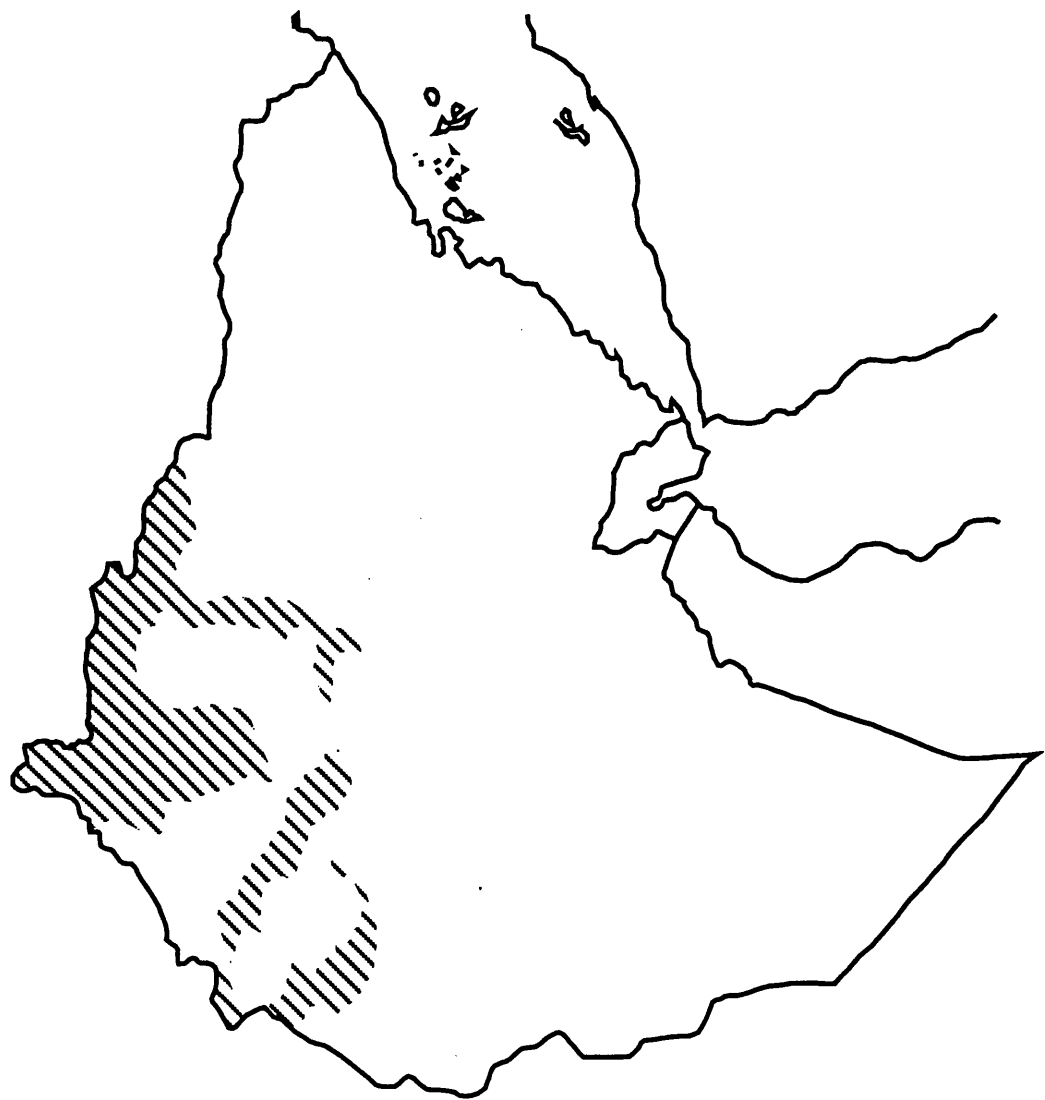
As a result of the investigation by NTTICC, different organisations and individuals, six species of trypanosomes have been recorded in Ethiopia and these are the most important parasites from both a medical and veterinary point of view because they include the causative agents of human sleeping sickness and the trypanosomiasis in livestock. All are prevalent in areas below 2000 metres above sea level (masl). The recognised trypanosomes belong to the three Sub-genera (*Dutonella*, *Nannomonas*, *Trypanozoon*). These species of trypanosomes are *Trypanosoma* (*T. vivax*, *T. congolense*, *T. brucei*, *T. evansi* (Langridge, 1976 and Krug, 1971); *T. equiperdum* (Zelleke, Shafo and Kelil, 1980) and *T. rhodesiense* (Hutchinson, 1971). In addition the non-pathogenic species *T. theileri* occurs in some parts of the country. In general trypanosomiasis is categorised into two types depending on their means of transmission. These are tsetse-transmitted and non-tsetse-transmitted. Tsetse-transmitted trypanosomiasis of domestic livestock caused by *T. congolense*, *T. vivax*, *T. brucei* has a wide distribution in the west and south-west parts of the country. The river valleys that cut into the highland plateau that form part of the Abi, Baro, Omo, and the Rift valley river systems provide suitable environmental conditions for a number of tsetse species. The distribution of *T. congolense* (Plate 1.1) and *T. brucei* (Plate 1.2) in Ethiopia is directly related to the distribution of the tsetse fly (*Glossina*). While *T. vivax* (Plate 1.3) which may be both tsetse and non-tsetse transmitted is found in the entire country except the dry northeast and the highlands (Dagnatchew, 1982). Human sleeping sickness caused by *T. rhodesiense* has been found within the

**Plate 1.1** *Trypanosoma congolense* Distribution in Ethiopia.



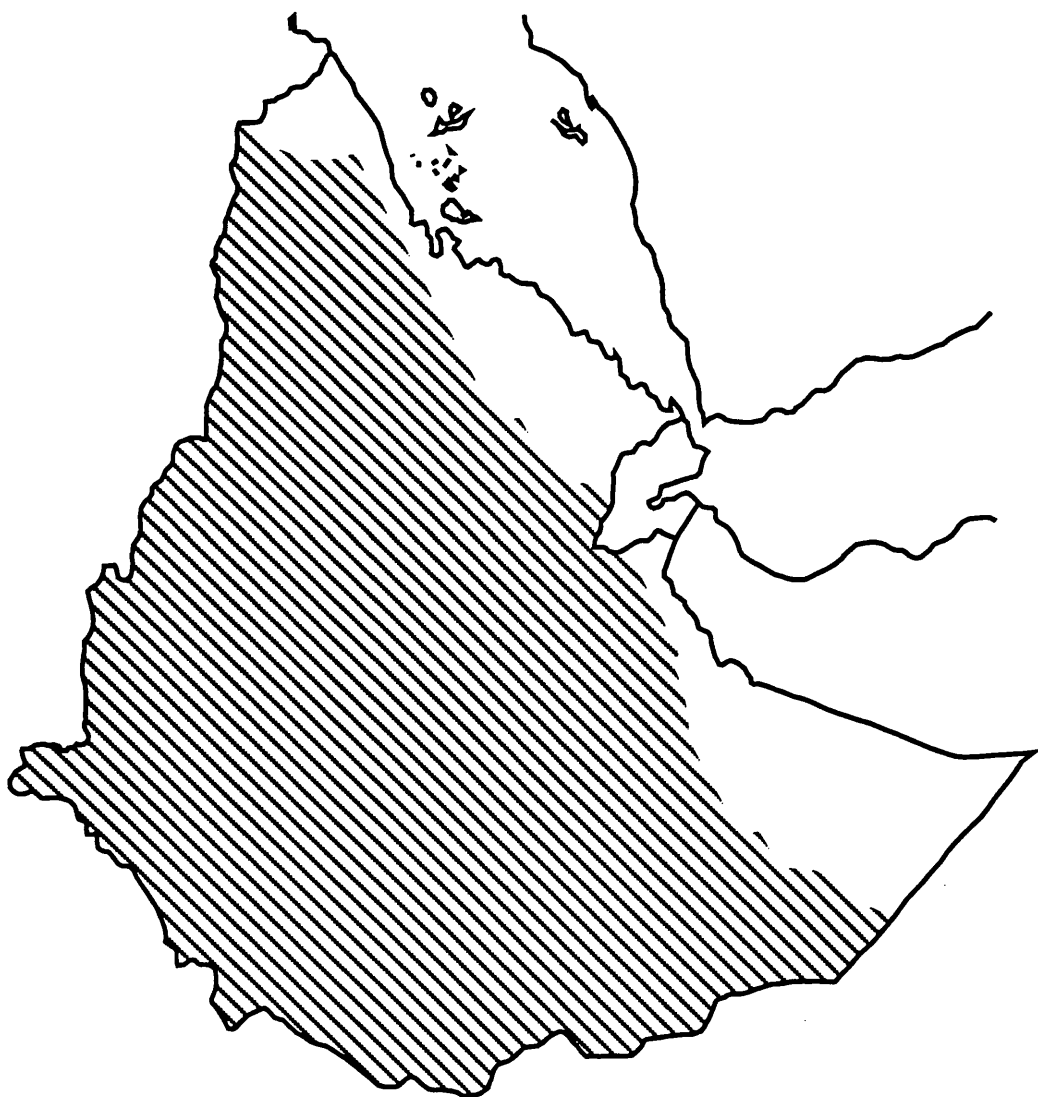
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**Plate 1.2** *Trypanosoma brucei* Distribution in Ethiopia



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**Plate 1.3** *Trypanosoma vivax* Distribution in Ethiopia



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tsetse belt mainly associated with the Baro river system. Periodic epidemics have occurred, related to cycles of drought which also plays a part in the epidemiology of animal trypanosomiasis. However, until recent time human sleeping sickness has not been a serious issue compared to animal trypanosomiasis. Non-tsetse transmitted trypanosomes include, in addition to *T. vivax* mentioned earlier, the mechanically transmitted *T. evansi* which is associated with camels in the south, east, and northeast and the sexually transmitted *T. equiperdum* of horses (Dagnatchew, 1982).

### **Extent and Distribution of Tsetse-flies (*Glossina*) in Ethiopia**

The genus *Glossina* occurs over some 11 million km<sup>2</sup> of Africa. Its northern limit extends across the continent from Senegal in the west to southern Somalia in the east. This limit is about 14° N but in Somalia it is only 4° N. The northern limit corresponds closely to the southern edges of the Sahara and Somalia desert. The southern limit is less well defined. In the southwest it varies between about 10° and 20° S, corresponding to the northern edges of the Kalahari and Namibian desert, whereas in the south-east it is generally at about 20° S but extends as far as 29° S along the east African littoral (Jordan, 1986). Thirty seven countries are affected. Tsetse flies are found only in Africa.

Tsetse-flies are classified into three subgenera which usually correspond with three principal ecological groups. These are:

The fusca group (subgenus *Austenina*) - the forest tsetse- are difficult to catch and find. They are of the least economic importance.

The palpalis group (subgenus *Nemobina*) - the riverine tsetse - are important vectors of human trypanosomiasis and some are important as vectors of animal trypanosomiasis.

The morsitans group (subgenus *Glossina*) - the savannah tsetse - are very important vectors of animal trypanosomiasis. They are widespread for most of the year, but retreat to denser woodland and other refuges during hot, dry periods.

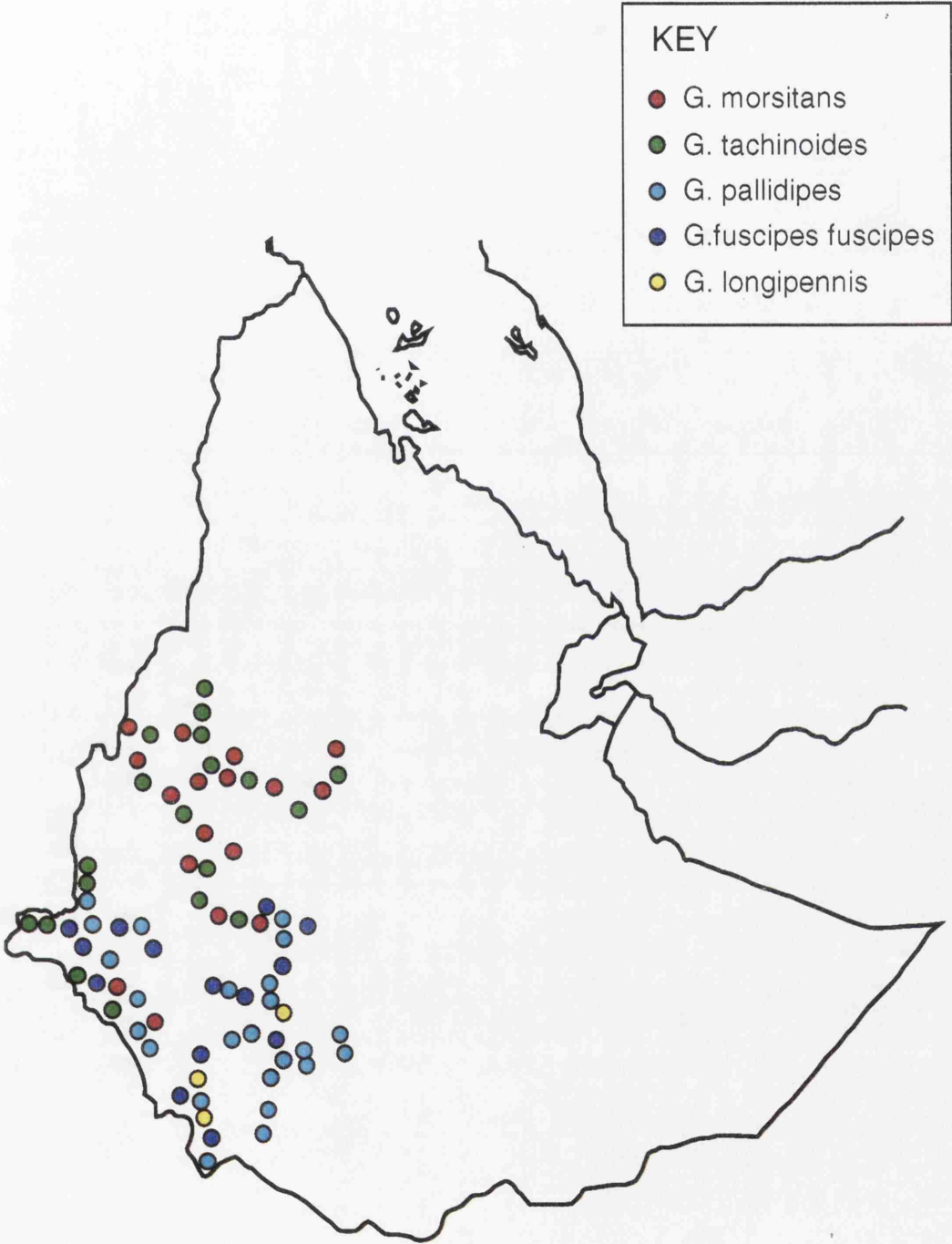
In Ethiopia tsetse flies and their impact has been recognised at national level for many years, but attempts to control them only started relatively recently compared with many other African countries. Different species of *Glossina* occupy a vast area of potentially arable land in the lowlands, where they have considerable socio-economic impact (Plate 1.4). The work reported by Langridge in 1976 indicated that some 98,000km<sup>2</sup> of the western and southwestern parts of Ethiopia were infested with tsetse. Since then the fly appears to have made major advances including an extension of the upper altitudinal limit. In 1988 it was estimated that the total area of the country infested was in excess of 120,000km<sup>2</sup> (FLDP, 1989). Fly progress has been mainly confined to the river valleys which penetrate into the western borders of the highland plateau and include the two savannah species *G. m. submorsitans* and *G. pallidipes*.

The species of tsetse flies (*Glossina*) found in Ethiopia include the three groups (morsitans, palpalis and fusca) (Ford and Katondo, 1977). They are found in the seven south western and western regions of Sidamo, Gamo Gofa, Kefa, Illubabor, Wellega, Gojam and western Shoa between longitude 33° and 38° E and latitude 5° and 12°N (Krug, 1971; Langridge, 1976; Fuller, 1978). They are associated with the major drainage systems of the Blue Nile / Abai / Baro, Omo and a few of the Rift Valley lakes. The two savannah species (*G. m. submorsitans* and *G. pallidipes*) are widespread whilst the two riverine species (*G. tachinoides* and *G. fuscipes*) are mostly confined to the main rivers showing a linear distribution pattern. Moreover *G. longipennis* is found in restricted areas in the drier lower Omo Valley (Langridge, 1976; Dagnatchew, 1982).

Expansion up the main rivers involves colonisation of valley floors and dispersal of flies into tributary systems and across watersheds. The most important spread in economic terms has been the invasion of tsetse into the densely stocked higher altitude areas (FAO, 1987).

The advance of *G. m. submorsitans* in the Abai river system and particularly

**Plate 1.4** Tsetse-fly (*Glossina*) Distribution in Ethiopia.



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in the Didessa valley, and *G. pallidipes* in the Omo river system, are relatively well documented. In 1966 this species could be caught up to 1300 masl (Balis and Bergeon, 1966). However, surveys carried out by Langridge (1974, 1976) indicated altitudinal limits of 1400 and 1600 metres above sea level respectively. More recently it was reported that *G. m. submorsitans* in the Upper Didessa valley were caught throughout the year up to 1700 masl and seasonally fly dispersal reached up to 2000 metres. It is assumed that recorded changes to the limit of fly distribution have not been significantly influenced by the development of better survey techniques since *G. m. submorsitans* is readily attracted to slow moving vehicles and would have been caught at higher altitude areas if present in earlier days. Additional evidence for the spread of *G. m. submorsitans* in the Abai river system is provided by governmental reports on the widening of the areas affected by trypanosomiasis and the rapidly increasing incidence of trypanosomiasis in domestic livestock on the perimeter of the highland plateau (NTTICC, 1987). In a year-round ecological study of *G. m. submorsitans* in the Fincha valley, using vehicle patrols and screen/handnet capture, it has been reported that the flies were distributed at 1600 -2000 masl which is far above the known altitude limit and at various intervals flies have been collected from the edges of the escarpment at an altitude of about 2200 masl. During the study period several pupae were collected at 2000 masl (Tikubet and Gemechu, 1984).

Although the current distribution of *Glossina* in Ethiopia is not clearly demarcated because of recent progress into some areas and disappearance from other areas, there is strong evidence that the following species are widely distributed in the western and southwestern part of the country.

#### ***Glossina morsitans submorsitans***

This species has an east to west distribution from Ethiopia to Senegal, occurring in vegetation often dominated by *Isberlinia* species (Jordan, 1986). The 1962 map which showed the presence of *G. m. submorsitans* in Ethiopia was made by Ovazza in 1956. However there were complications over the identification of the



subspecies and later Balis and Bergeon stated they were, in fact *G. m. submorsitans* (Ford, 1971). The species is widely distributed in the savannah woodland and grassland areas (Ford and Katondo, 1977). It is found north west of Gambella and south of the Akobo river on the Sudan/Ethiopian border. It is thought to be present at the headwaters of the Baro river and in most of the western lowlands (Krug, 1971). It has a relatively restricted distribution in the Omo river basin east of the Sobat drainage. In the Omo basin it was found as far north as the confluence of the Omo and Dancia rivers over to the southern base of the Maji mountains. The species is not abundant east of the Maji mountains (Fuller, 1978). The advance of this species was also noted in the Muger Valleys (Chater, 1983). In the Anger and Didessa Valleys it is found in grassed woodland, bamboo forest and marshy grassland. The natural area at the Batch had the highest apparent density and the oldest male flies; while the bamboo area had the lowest apparent density and the youngest male flies. None of the localities showed any marked changes from one season to another. The highest proportion of females and teneral flies were caught in the bamboo, the lowest proportion were found in the oxen area (Bourn and Scott, 1978). In the Upper Didessa Valley *G. m. submorsitans* occupies the woodland and, the floor and side of the valley. It is only during the short dry season, after bushfires, that this species seeks the shelter of vegetation on the drainage lines (Slingenbergh, 1992).

Hutchinson, Balis and Bergeon as cited by Fuller (1978) reported the presence of *G.m. submorsitans* along the Mui river and around Lake Abaya in the Rift valley. Besides that it is widespread in the Upper Sobat, the Blue Nile drainage system and north and south of the Akobo river. It is an important vector of human sleeping sickness diagnosed in the Akobo-Gilo area. The hamlet of Pinybago is in dense savannah woodland and *G. m. submorsitans* is present (Jordan, 1986). In the Ghibe valley of western Ethiopia *G. m. submorsitans* was first captured in 1989. Since then occasionally a number of flies have been caught, although infection was not detected amongst dissected flies (Leak *et al.*, 1993). However the overlap of the two species

in this part of the country is significant.

### **Glossina pallidipes**

This species occurs in East Africa from Ethiopia to Mozambique in a range of vegetation types and climatic conditions (Jordan, 1986). In Ethiopia it is particularly common along the Omo river where it was traced in a continuous band from 20km above Lake Rudolf to the Jimma/Addis Ababa road. It was found along the southern edge of the plateau from Lake Chamo in the Rift Valley to the Sobat and Blue Nile drainage basins. The species is also common on the Sagan river. It is also present, but less common across the low-lying plateau between the Omo river and Sobat drainages. It was not found in the extreme south where a semi- desert climate prevails (Fuller, 1978; Ovazza and Rodhian, 1972).

McConnell *et al.* as cited by Fuller (1978) indicated the presence of *G. pallidipes* on the Gojeb river, a large tributary of the Omo. In the south and east of the Maji mountains, the predominant species away from the gallery forest was *G. pallidipes*. According to Krug (1971) it has been identified near Gambela, at the headwaters of the Gilo river and in the centre of the Omo river valley.

In the Ghibe valley (Ghibe and Tolley) it is the predominant species. The mean relative densities of the tsetse caught, from March 1986 to April 1990 was 1.42 and 5.58 respectively. The variation in tsetse density appeared to be the main factor responsible for variation in tsetse challenge and trypanosome prevalence (Leak *et al.*, 1993). Its advance up the Omo valley has been recorded (Chater, 1983).

### **Glossina tachinoides**

In the Didessa valley this species of tsetse is found in the dense vegetation of the main river and rarely disperses far along the tributaries or drainage lines where the evergreen vegetation is less dense (Slingenbergh, 1992). Similarly in the Angar Didessa valleys it is found in the riverine forest (Bourn and Scott, 1978). Although it was not found in the Omo basin during the last survey, it has, previously been collected in the Sobat basin along the Gilo, Baro and Akobo rivers, as well as along

the Didessa river in the Blue Nile basin and it has also been collected just east of Lake Abaya (Lake Margharita) in the Rift valley nearly 400km from the nearest collection site to the west. Since conditions in the Omo basin are comparable to *G. tachinoides* habitats in the Sobat basin and the Lake Abaya areas, it is possible that this species also exists there (Fuller, 1978; Ovazza and Rodhian, 1972). It has been implicated as a major vector in human sleeping sickness in south west Ethiopia (Hutchinson, 1971; Buyst, 1977; Bekele, 1975; Nozais, 1985 and Jordan, 1986). Ovazza, Balis and Bergeon as cited by Ford (1971) and noted that this species occurs together with *G. fuscipes*.

### **Glossina fuscipes**

This was the dominant species found on the Omo river from the Jimma/Addis Ababa road to within 20km of Lake Rudolf. It is also found along tributaries of the Omo, such as the Mago, the Gojeb and the Mui rivers and in the gallery forest along rivers draining the western escarpment in the southern part of Ethiopia. When there was a rise in the number of crocodiles, a drop in the number of *G. fuscipes* was observed, but this drop in the number of *G. fuscipes* corresponded more closely to the drop in numbers of hippopotami and suggests a greater dependence on hippopotami than on crocodile as a food source (Fuller, 1978). In contrast (Hoare, 1962) noted that along the Upper Nile, crocodiles and lizards are the main source of food for this species. Weitz (1963), as a result of blood meal identification in East Africa, stressed the importance of reptiles and did not include the hippopotamus as a potential alternative host for *G. fuscipes*. It has also been identified west of Gambela, near Dembi Dolo, at the Gilo and Akobo rivers, north of Lake Rudolf, and in the Upper Omo river valley (Krug, 1971).

In the Ghibe valley (Ghibe and Tolley) very few *G. fuscipes* were captured at either site. However, the numbers captured at Ghibe increased steadily from an annual mean relative density of 0.1 flies /trap per day in 1986 to 0.6 flies/trap per day in 1990 (Leak *et al.*, 1993). This species was also implicated in the transmission of

sleeping sickness in Illubabor in the western part of the country in 1967 (Bekele, 1975; Buyst, 1977).

### **Glossina longipennis**

This species is found in the savannah from the base of the Maji mountains across the Mui game park and along the Omo river to the base of the Hamar plateau east of Kerre. This is an arid to semi arid savannah tract, which supports many game animals (Fuller, 1978). Lewis; Balis and Bergeon; Hutchinson as cited by Fuller (1978) *G. longipennis* has been collected across southern Ethiopia from Somalia into the Sudan, although its distribution appears to be patchy. It was reported by Ghidini to be found in the Rift valley as far north as the Sodo area. According to Krug (1971) this species has been identified at various places in the south, mainly in the lower Omo river valley.

### **Glossina bravipalpis**

Although Ghidini (1938) reported that *G. bravipalpis* was present in the lower Omo, many investigators (Langridge, 1976; Ovazza and Rodhain, 1972; Fuller, 1978) failed to confirm this. Fuller (1978) postulated that it might possible that this species was present at one time, but that with the destruction of most large game in the lower Omo it too disappeared; perhaps it still inhabits the area but is not attracted to man. Ford *et al.* (1976) mentioned in their report to the government of Ethiopia that *G. longipennis*, *G. bravipalpis*, *G. pallidipes*, and *G. austeni* were found lower down the Juba valley in Somalia about 400 km. away from the Ethiopian border. Although the reason is obscure, it appears that *G. longipennis* has disappeared from the Genale basin. Nevertheless, there is a possibility of advancement or incursion of some of the flies into Ethiopia in the future.

Overlaps in the distribution of the Morsitans group (*G. m. submorsitans* and *G. pallidipes*) and the palpalis group (*G. fuscipes* and *G. tachinoides*) have been recorded in the western part of Ethiopia. These areas of overlap are areas in which trypanosomes are most liable to be transferred from wild animals to man and his

livestock and for this reason they have a profound epidemiological and epizootological significance (Ford, 1971).

### **Factors Influencing Tsetse Distribution**

Early studies indicated that tsetse distribution is determined by the climate, and secondarily by vegetation, which can mitigate the severity of the climate (Nash, 1969; Ford, 1971). In south-eastern Africa, where the rainfall is high and there are no deserts, the limit of *Glossina* is determined by seasonal low temperatures. This may affect the adults which are inactive below about 16°C, or so lengthen the period spent in the soil as puparia, that all the contained fat is used up before development of the adult stage can be completed (Bursell, 1960).

#### **Climate**

There is a wide range of climates in Ethiopia and this is dependent upon the altitude of the area. The climate becomes progressively more arid to the south of the Maji mountains where there is a double rainy season. In the far south of Lake Rudolf, the altitude is only slightly over 400 metres, and in the west along Akobo, the altitude is 600 - 700 m. The altitude gradually increases towards the Ethiopian plateau to the north and east (Fuller, 1978). Welde Mariam; Butzer as cited by Fuller (1978) mentioned that the southern and western edge of the plateau rises from 2000 - 3000 m. The Maji mountains between the Omo and Akobo rivers, rise to around 2000 m. The rainfall increases dramatically with altitude from well under 500 mm per year at Lake Rudolf to over 1600mm per year at Maji and over 1800 mm per year along the western escarpment.

The Ghibe valley has a pronounced dry season from November to February with rainfall occurring between March and April and from late May to October. The mean maximum temperature ranges from 29.8°C to 44°C. Temperatures are generally lower from June to August and highest from October to May (Leak *et al.*, 1993). In the Angar Didessa valley although there are no long-term climatic records

for the area, the information that is available indicates an annual rainfall between 1300- 1500mm, with a six month wet season from May until October and a dry season from November until April. Mean maximum monthly temperature range from 7.8°C (August) - 38.3°C (April). Mean minimum monthly temperatures range from 7.8°C (December) - 19.6°C in May (Bourn and Scott, 1978).

### **Habitat**

Apart from suitable climatic conditions tsetse flies are dependant on the presence of woody vegetation for shelter and host animals to feed upon. Though the abundance of animals (wild or domestic) may play a role in tsetse population dynamics, the availability of host animals is not believed to influence the general picture of fly distribution. Vegetational patterns are more important in this respect especially where as a result of human activity the environment has changed to such an extent that it has become less inhabitable to the two savannah species *G. m.submorsitans* and *G. pallidipes*. As a general rule both species are widely dispersed in the humid savannahs of the western part of the country.

### **Vegetation**

Pratt and Gwynne as cited by Bourn and Scott (1978) have described in some detail the Angar Didessa river system which is a tributary of the Blue Nile (Abbai). This is representative of most areas in the Blue Nile river system and has four major vegetation types within the valley: grassed woodland, riverine forest, marshy grassland and lowland bamboo forest. The dominant type of vegetation is combretaceous woodland. The most common trees are *Combretum* spp., *Terminalia*, *Philostigma*, *Albiza*, *Gardenia*, *Grewia* and *Stereospermum* with hyperrhenia grass in between. At the end of wet season this grass can reach 3-4 metres in height. Dense evergreen riverine forest occurs along even the smallest of streams. The drainage lines, as seen in aerial photographs, are thus conspicuous and have a markedly dendritic appearance. A rich variety of plant species occurs in these humid forests. Poorly drained lands which are marshy during the wet season are

characterised by open areas of grassland, often bounded by distinct lines of trees, shrubs and thicket vegetation. Thickets also occur in the combretaceous woodland in association with termite mounds and fig trees. The fourth vegetation type, lowland bamboo forest is an unusual feature of the region. Dense almost pure stands of *Oxtenanthera abyssinica* cover particular hillsides and well drained rocky ground.

As has been indicated the distribution of the various vegetation types is greatly influenced by water availability and drainage, but fires also play an important role. Indeed, it is generally accepted that combretaceous woodland is a fire induced climax vegetation. During the dry season, bush fires rage through the valley with increasing frequency and intensity. The humid evergreen riverine forests act as effective fire breaks and, except for the outer vegetation they do not appear to be seriously damaged (Bourn and Scott, 1978). In the Akobo, Omo and Segen river basin in Keffa, Gamo Gofa, Sidamo, and Shoa regions, acacia and short grass steppe are dominant. The flora is dramatically influenced by proximity to the plateau (Fuller, 1978).

Many areas under 1500 masl are potential tsetse-fly country. Vegetation varies from semi-desert to tree and shrub steppe, savannah forest or treeless grassland, with arable land mostly limited to a few small maize fields along the rivers. This includes the valleys of Omo, Gojeb, Didessa, and Baro (including the headwaters), and the Gilo and Akobo (Krug, 1971; Langridge, 1976).

### **Wild animals**

The role of various species of African wild animals as reservoirs of salivarian trypanosomes have been reviewed by Ashcroft (1959) and Lumsden (1962). It has long been known that the distribution and abundance of at least some *Glossina spp.* are closely related to the abundance and habits of wild animals (Jordan, 1986).

It is well known that the southern and western provinces are Ethiopia's main game areas, but the density and distribution varies considerably. In the large river valleys of the northeast (valleys of the Omo, Gojeb, Didessa and upper Baro rivers),

the red buck (*Redunca reduncia*), the bush buck (*Tragelaphus scriptus*), the warthog (*Phacochoerus aethiopicus*) and the bushpig (*Photamocheerus porcus*) are found. The buffalo (*Syncerus caffer*), also inhabits parts of the Omo, Gojeb and upper Baro river valleys (Krug, 1971). According to the report of the FAO/WHO expert committee on African trypanosomiasis, all the above mentioned species are important hosts for tsetse fly and may be regarded therefore as reservoirs of trypanosomiasis (FAO, 1979).

In the southern tree shrub steppe of the rift valley, all the aforementioned species are found, as well as various species of plains game, such as Burcelle's and Grevy's zebra (*Eguus burchelli*, *E. Grevyi*), Grant's gazelle (*Gazella granti*), hartebeest (*Alcelaphus buselaphus*), Tiang (*Damaliscus doracus*), Oryx (*Oryx beisa*), and Giraffe (*Giraffa camelopardalis*). In the west, the Giant foresthog (*Hylochoerus meinertzagani*) inhabits the rainforest and elephants (*Loxodonta Africana*) occurs in small numbers on the savannah plains.

Areas with numerous game animals are generally those in which occurrences of tsetse fly are known or suspected. In fact, in the western savannah plains and in parts of the south, tsetse fly depend entirely on game animals for survival, as no domestic animals are kept. Tsetse blood-meal analysis results and observations showed that in the Fincha valley suids, bovids and others were the most common hosts (Tikubet and Gemechu, 1984).

### **Tsetse-flies as Vectors of Trypanosomes**

Infection rates of trypanosomes in *Glossina* vary from species to species and from one locality to another. The early data were reviewed by Buxton (1955) and more recent studies indicate that a variety of factors can influence the establishment of infections by salivarian trypanosomes in *Glossina*. These have been reviewed by Jordan (1976) and by Molyneux (1977). These factors are identified as: a) endogenous factors associated with the fly, b) ecological factors, c) parasite and host



(Molyneux, 1977).

In Ethiopia studies indicate that mature trypanosome infections in dissected tsetse are 3.6% in *G. pallidipes* and 1.2% in *G. fuscipes* at Ghibe, and 6.2% in *G. pallidipes* at Tolley (Leak *et al.*, 1993). Whereas in *G. m. submorsitans* the overall infection rate of *T. vivax* and *T. congolense* (no *T. brucei* detected) in the three areas where 1049 dissections were performed, was 8.6% (Bourn and Scott, 1978).

In similar studies in Somalia the infection rate of *G. pallidipes* was 2.6% and 1.5% in the wet and dry season respectively (Mohamed Ahemed and Dirie, 1987). The work conducted in the Bahr El Arab, south Darfur province of Sudan showed that the overall infection rate of *G. m. submorsitans* captured in the four areas was 5.1%. *T. vivax* comprised 64.7% of the total infections, *T. congolense* 31.2% and *T. brucei* 3.9%. 23.7% of the *T. vivax* and 31.3% of the *T. congolense* infections were immature and mixed infections were encountered in a few flies (Mohamed Ahemed *et al.*, 1989).

In West Africa the highest trypanosome infection rates in wild tsetse were in *G. m. submorsitans* caught close to routes along which herds of heavily infected trade cattle, regularly passed (Jordan, 1965; Baldry, 1969; Riordan, 1977).

### **Tsetse-transmitted Trypanosomiasis**

The salivarian trypanosomes are natural parasites of many species of African wild animals, which acquire symptomless infection and long-lasting parasitaemias. All species of domestic animals are susceptible to infection with one or more species of the salivarian trypanosomes. The pathogenicity of each trypanosome species varies in different host species and the course of a trypanosome infection can be influenced by the size of the infecting dose, which tends to be variable under natural conditions of transmission (Holmes *et al.*, 1979; Murray, 1984; Losos, 1986).

Although most species of trypanosomes can cause serious disease in their hosts, cattle trypanosomiasis as elsewhere in Africa appears to be the most important

from an economic point of view since the agricultural sector in this country is fully dependent on animal traction particularly by oxen. For this reason the review starts with cattle trypanosomiasis.

### **Tsetse-transmitted Trypanosomiasis in Cattle**

Three distinct species, belonging to different subgenera, are pathogenic in cattle. These are *Trypanosoma (Nannomonas) congolense*, *T. (Dutonella) vivax* and *T. brucei* which belongs to the subgenus *Trypanozoon*. All three species are found in cattle throughout the tsetse-infested areas and it is not uncommon to find mixed infections (Losos and Ikede, 1972). The three species can be distinguished on morphological grounds and by characteristic behaviour when observed on a fresh wet blood film. When examining blood samples from cattle in endemic areas of trypanosomiasis, *T. congolense* and *T. vivax* are usually found more frequently than *T. brucei* and in general are considered to be more pathogenic. However, after subinoculation of blood into laboratory animals, a much higher incidence of infection with *T. brucei* may be found (Anon, 1986). The former two species, cause by far the greatest economic losses and *T. congolense* is generally regarded as the most important form of animal trypanosomiasis in east Africa (Losos and Ikede, 1972).

Recent studies indicated that the advance of *G. m. submorsitans* in the Didessa valley of western Ethiopia resulted in an exponential increase in the annual trypanosomiasis incidence in livestock even at the 1900-2000 masl. The period between disease transmission and diagnosis is about one month, and monthly disease incidence was found to be strongly correlated with the mean daytime temperature of the preceding month. The first survey, carried out in 1986, revealed that 60% of the cattle were positive for trypanosomiasis. The disease had affected each and every house-hold (Slingenbergh, 1992). Whereas the prevalence of trypanosomiasis in Buno province involving six districts was found to be 18 % (Asfaw, 1986). In Bedelle veterinary clinic alone five cases of haemorrhagic *T. vivax* infections were

diagnosed between October 1985 and April 1986 and it is still not known whether this form of *T. vivax* infection is confined to tsetse infested areas only or whether it is also present in tsetse-free areas of the country (NTTICC, 1986).

On the Arjo escarpment studies revealed that the prevalence of trypanosomiasis in cattle was 13% with *T. congolense* accounting for 74% of the infections followed by *T. vivax* at 24%. One case each of *T. brucei* and *T. theileri* were detected (Bekele, 1992). Earlier studies in this area had shown 23% trypanosome positivity on thin and thick blood film examination with *T. congolense* showing in 80% of cases (Scott and Pegram, 1974). The preliminary study on the prevalence of bovine trypanosomiasis in the Nekemte province of the Wellega administrative region revealed infection levels of 32% out of which *T. congolense* and *T. vivax* were found to be 46% each. Mixed infections were 8 % (Sori, 1986). In June 1972, work oxen were introduced into the Angar Gutin settlement and one month after the first 40 oxen arrived a 100% infection rate was detected. All appeared to be *T. vivax* infections except for one *T. brucei* and one *T. congolense*. Since then high overall infection rates were almost invariably found just prior to retreatment, but the proportion of *T. vivax* infections declined whilst those of *T. congolense* increased. The ratio was about 90% of *T. congolense* : 10% *T. vivax*. Only three *T. brucei* infections were identified out of a total of 1816 blood samples examined. Comparison of fly and oxen infection rates were of considerable interest. Over the year of study 10 % of tsetse examined were infected with trypanosomes while 50-60% of the oxen usually became infected just prior to treatment. However, the ratio of *T. vivax*: *T. congolense* infections was reversed, 5 : 2 in tsetse and 1 : 9 in oxen (Bourn and Scott, 1978). The number of animals reached 450 by 1977. However, by 1979 only seven of the draught oxen remained alive because of the decline of management and disease control (Jordan, 1986). In addition great losses among cattle in Illubabor region along the Baro river around the highlands of Dembidolo have been recorded due to *T. congolense* and *T. vivax* which were

identified more frequently than *T. brucei*. Trypanosomiasis has been recorded as the main problem in most areas of Kaffa, Illubabor and Wellega regions (following the main river valleys) (Krug 1971). In Gimira province of the Kaffa administrative region the trypanosome prevalence in Sheko and Zebu types of cattle, maintained together, were 14.6% and 17.1% respectively. The mean prevalence of the two groups was 16.2%. During this study the trypanosome species encountered were *T. congolense*, *T. vivax* and *T. brucei*. They accounted for 34.4%, 31.2% and 8.6% and mixed infections for 25.8% (Abebe, 1992).

The survey conducted in the Wenago province of the Sidamo administrative region showed that 16.6 % of randomly selected cattle were positive for trypanosomiasis. In addition, out of 1616 animals brought to Dilla veterinary clinic for examination 510 (32%) were found to be positive. The species of trypanosome identified during this study included *T. congolense*, *T. vivax* and *T. theileri* with proportional prevalence rates of 59%, 33%, and 1%. Mixed infection of the first two accounted for 5.%. In Welaita province a survey revealed an infection rate of 6.4%, with *T. vivax* the most prevalent species (Jemal, 1986). In the Gamu Gofa administrative region it was noted that 12.7% of randomly sampled cattle were positive for trypanosomes. The identified species were *T. congolense*, *T. vivax* and *T. brucei* which accounted for 71%, 21% and 4% respectively and with 5% mixed infection (Elos, 1990). In the Kindo Koisha area a survey carried out by the NTTICC in 1992 showed a prevalence of 16.7% infection among randomly sampled cattle, but in the Makai valley (Southern Omo) the prevalence was 8% (NTTICC, 1990).

A survey conducted from October 1984 to May 1985 in 37 villages of the three provinces of the Gamu Gofa region of south-western Ethiopia showed an infection level of 32% in zebus: *T. congolense* was the most common species, followed by *T. vivax*, *T. brucei*, and *T. theileri* (Takele and Abebe, 1988; Abebe, 1987). Likewise the studies carried out in the Ghibe valley (Ghibe and Tolley) where

the area is infested with three species of *Glossina* indicated that *T. congolense* was identified in an average of 81% of the blood samples collected from parasitaemic adult cattle in 1986, 1987 and 1988. The percentage increased to 94% in 1989. In contrast, the percentage of samples containing *T. vivax* decreased from a mean of 16% between 1986 and 1988 to 7% in 1989 in the same group. The ratio of *T. vivax* to *T. congolense* infections was higher in animals younger than 24 months of age. *T. brucei* was responsible for very few cases of trypanosome infection, an average of 4% (Rowlands *et al.*, 1993). Previous work carried out by Minda (1991) in the Ghibe valley revealed that 18% of animals were positive for trypanosomes. The relative frequency of trypanosomes, *T. congolense*, *T. vivax* and *T. brucei* was 74.6%, 14.2% and 6.3% respectively. A study conducted by NTTICC in 1991 in Tolay shows that a prevalence of 51.7 %. Terefe (1993) reported that in the western Gojam region which includes Bahirdar and Kola Dega Damot the prevalence of trypanosomiasis in cattle was 13.2% in 1040 animals, out of which only two cases were due to *T. congolense*, while the rest were *T. vivax* infections. Similarly a survey conducted by NTTICC in 1992 in the Tana Beles project area revealed that 13% of the sampled cattle were found to be infected.

The severity of the disease in infected animals shows considerable variation from acute to chronic forms. In general *T. congolense* is the most important trypanosome affecting cattle in Africa. There are many different strains of this organism and they vary considerably in their virulence. In West Africa, trypanosomiasis caused by *T. congolense* is generally regarded as being a relatively mild, chronic infection whereas in East Africa it may be seen as an acute, subacute or mild chronic infection (Stephen, 1970). During the initial phase which lasts 4-6 weeks there is intermittent pyrexia, increased respiratory and heart rate, depression, pale mucous membranes, subcutaneous oedema of the jaw, and a prominent vagular pulse. The appetite is depressed and there is a loss of weight. Death may occur during this phase as a result of severe anaemia and circulatory collapse. Provided

cattle survive the initial phase or do not become reinfected, they gradually pass into the chronic stage of the disease which may be protracted and end in death or recovery. During the chronic phase there is a progressively decreasing parasitaemia and parasites become difficult to detect or disappear completely from the blood. Despite the apparent absence of trypanosomes, in many cattle the anaemia persists and affected animals usually respond very poorly to chemotherapy. During the terminal stage the animal becomes recumbent for 1 to 3 days. The chronic phase may last for months, resulting in extreme emaciation and severe anaemia.

Cattle infected with *T. vivax* may show various forms of clinical syndrome. Hyperacute forms are characterised by a fulminating septicaemic-like syndrome with extensive haemorrhages on mucous membranes, bloody nasal discharge, and blood in the faeces. Death rapidly follows. In the more commonly observed less severe, but still acute syndrome haemorrhages are only occasionally observed. The high peaks of parasitaemia are associated with elevation of body temperature and death often occurs during these periods. The lymph nodes are enlarged, the mucous membranes are pale and there is a rapid loss of weight and appetite. The chronic syndrome is characterised by waves of parasitaemia, but at lower levels than in the acute syndromes. Extreme emaciation and subcutaneous oedema of the throat are commonly observed. Death in both acute and chronic syndromes is often sudden and not preceded by recumbency (Losos, 1986). There is a considerable evidence that cattle infected with trypanosomiasis particularly with *T. congolense* show a depressed immune response to foreign antigens (viral or bacterial) following vaccination (Holmes *et al.*, 1974.; Sharpe *et al.*, 1982 ; Rurangirwa *et al.*, 1980). The study conducted in Ethiopia on experimentally and naturally *T. congolense* infected zebu cattle which showed depressed immune response to polyvalent clostridial vaccine (Holmes *et al.*, 1974), to louping-ill virus vaccine (Whitelaw *et al.*, 1979), and to foot and mouth and clostridial vaccine (Scott *et al.*, 1977). This indicates that the animal health problems associated with trypanosomiasis might be further complicated by

decreased immunity to secondary infections and a reduced response to vaccination.

### **Tsetse -transmitted Trypanosomiasis in Sheep and Goats**

In a review of trypanosomiasis in sheep and goats Griffin (1978) indicated that many of the accounts of naturally occurring trypanosomiasis in sheep and goats came from East Africa caused by *T. congolense*. Although the epidemiology of trypanosomiasis in sheep and goats in the tsetse-infested areas of Ethiopia is not well documented there is evidences that sheep and goats succumb to the disease. In addition studies conducted in neighbouring countries with similar ecological situations indicate that levels of infection in small ruminants can be significant. A study conducted in various breeds of sheep and goats in Kenya indicated that exotic breeds were more susceptible to natural trypanosomal infection than indigenous breeds and that the infection may be severely debilitating and in many cases fatal. In the Kenyan study an increase in tsetse numbers which occurred one month after substantial rainfall, was followed by an increase in the prevalence of trypanosomiasis in small ruminants (Griffin and Allonby, 1979). Limited studies in Ethiopia have detected trypanosome infections in small ruminants. In the Welaita province of the Sidamo region it was demonstrated that trypanosomiasis infection in goats was 4.8% (Jemal, 1986). Whereas an investigation carried out in the three administrative regions of Kaffa, Wellega and Illubabor showed an infection rate of 2.4% in sheep and 2.7% in goats (TCS, 1983).

Most of the reports of experimental trypanosomiasis in sheep and goats concern *T. congolense* and *T. vivax*, (Losos and Ikede, 1972). A study conducted in Somalia to investigate the effects of trypanosomiasis on 40 sheep in a tsetse-infested area showed that 60% eventually developed trypanosomiasis out of which 71% was due to *T. congolense* (Dirie *et al.*, 1988). The clinical signs are similar to those seen in cattle, where three syndromes have been described, namely acute, subacute and chronic (Griffin and Allonby, 1979). These animals usually suffer from a subacute or

chronic form of the disease (Soltys and Woo, 1977). The course of naturally acquired *Trypanosoma congolense* infections in two breeds of sheep and two breeds of goats was monitored in an endemic area of Kenya. The first two types, the acute and subacute, were sub-divided on the basis of the outcome of the disease, which was either fatal or self-cure, but the third type, the chronic form, invariably ended fatally (Griffin and Allonby, 1979).

Infections in sheep are characterised by progressive anaemia, loss of weight, debilitation, oedema, and enlargement of lymph nodes, with neurological signs and ocular involvement later in the infection, and high mortality (Ikede and Losos, 1975). The different lesions occurring in the disease are largely determined by the distribution of the parasites in the host. The *brucei* group causes extensive degenerative changes in the tissues of the sheep and goats as it does in cattle, with anaemia of secondary importance (Losos and Ikede, 1972). Severe ocular lesions are associated with the presence of trypanosomiasis in sheep infected with *T. brucei* (Ikede, 1974). *T. vivax* and *T. congolense* appear to exert their pathogenic effect mainly by the anaemia produced. Studies of the pathology of *T. vivax* in goats confirms that this parasite is largely restricted to the blood system (Van den Ingh *et al.*, 1976). In the course of infection with experimental *T. congolense*, infected sheep develop anaemia, the onset of which follows the first wave of parasitaemia. The changes in the blood lipids observed in infected sheep appeared to be related to the intensity and duration of parasitaemia (Katunguka -Rwakishaya, Murray and Holmes, 1992).

### **Tsetse-transmitted Trypanosomiasis in Horses**

Three species of trypanosomes (*T. brucei*, *T. congolense*, *T. vivax*) are responsible for equine trypanosomiasis in Africa. All three cause fatal disease of the same nature, differing only in the fact that the course of infection by tsetse transmitted *T. brucei* is generally rapid and usually fatal, whereas the illness caused by the other



two is more frequently chronic (Hornby, 1919). Donkeys and mules appear to be less susceptible than horses. The earliest sign of the disease in horses, donkeys and mules is a stumbling gait and a harsh coat. As the disease progresses, subcutaneous oedema may appear on the ventral parts of the body, hind legs, and genitalia. With the progress of anaemia, the mucous membranes of the eye become gradually paler. Lymphadenitis is usually present, but the superficial lymph nodes in the horse are not markedly enlarged (Soltys and Woo, 1972). *T. congolense* and *T. vivax* tend to cause mild and chronic disease, characterised by weakness, loss of condition, anaemia and subcutaneous oedema. Infected animals may recover spontaneously although severe fatal infections have been reported.

In the Didessa valley of western Ethiopia after the tsetse invasion, equines appeared to be the most susceptible livestock and died first, followed by cattle (Slingenbergh, 1992). In Welaita province the prevalent species of trypanosome in equines was *T. brucei* which infected 6.4% of the sampled animals (Jemal, 1986). In earlier observations *T. vivax* had been identified most frequently in equines, but the pathogenicity was moderate, whereas *T. brucei* was mainly responsible for severe losses among equines in the southern part of Keffa province (Krug, 1971). An experimental fly transmitted infection caused by *T. congolense* in the horse showed a moderate degree of anaemia. The lowest red cell determination was recorded one month after the infection began. Parasites were never seen in large numbers in the peripheral blood and the animal remained alert and lively with other signs of infection such as oedema of the legs were not striking enough to cause concern (Stephen, 1986).

### **Tsetse-transmitted Trypanosomiasis in Camels**

Because of their geographical distribution, camels are seldom exposed to tsetse challenge, but they are susceptible to all the tsetse-transmitted trypanosomes and suffer severe disease if infected. *T. vivax* infection may run an acute and fatal course but it is usually less severe than *T. congolense* (Hornby, 1952). Martoglio as

cited by Stephen (1986) reported an acute disease in camels in Eritrea in Ethiopia which was almost certainly due to *T. vivax*. Similarly Wenyon as cited by Stephen (1986) reported that a convoy of over seventy camels taken into the Bahrel Ghazal province of the Sudan all died within a period of two months following infection with *T. brucei*.

### **Tsetse-transmitted Trypanosomiasis in Humans**

The first case of sleeping sickness in Ethiopia was reported in 1967 at the Gilo river mission station in Illubabor province. Before that time there were no records of the disease. Since 1967 an increasing number of patients have been observed at several places. *Trypanosoma rhodesiense* was identified, and it was associated with the occurrence of *G.m. submorsitans*. It was speculated that refugees from the Sudan crossing into Ethiopia might have carried the disease. Because of the very few health stations in that area, all run by mission personnel, and because of the vast distances and complete lack of roads, only a small number of cases may have been treated. Often patients arrive at mission stations after weeks of travel on foot (Krug, 1971).

Epidemiological studies indicate that human trypanosomiasis caused by *Trypanosoma brucei rhodesiense* has become a problem along the nearby Gilo, Akobo, and Baro rivers (Baker *et al.*, 1970; McConnell *et al.*, 1970). During the closing months of 1968 the first cases appeared among women and infants, indicating that transmission was beginning to occur in the vicinity of permanent settlements. This suggested that the risk to the population as a whole was increasing and was no longer restricted to hunters, travellers or honey collectors, the traditional victims of sporadic Rhodesian sleeping sickness (McConnell *et al.*, 1970). It was estimated that 50 - 100 deaths probably occurred before people began to seek treatment. *G. m. submorsitans* almost certainly transmitted the infection from wild animals to man, and there is a strong circumstantial evidence that *G. tachinoides* and to a smaller extent *G.*

*pallidipes* were vectors of the village foci (Hutchinson, 1971; Nozais, 1985; Bekele, 1975). The centre of infection appeared to be on or near the Gilo river, though difficulties of communication preclude the possibility of knowing the situation in the area from the Gila River southward to the Akobo River. Most of the inhabitants of this region live in small, discrete river-side villages, situated in incompletely cleared areas in the dense riverine woodland (McConnell and Baker, 1970). Along the Gilo river in the south-western part of the country, in Illubabor province, 4 cases were confirmed and 28 diagnosed in 1968, 173 in 1969, and 42 in the first ten months of 1970 (McKelvey, 1973). There have been sporadic cases since then but these are not well documented.

Research findings have indicated that domestic animals act as reservoirs of causative agent of African sleeping sickness in both east and West Africa (Gibson *et al.*, 1978). In the Gamo Gofa province of south-western Ethiopia a strain of *T. brucei* isolated from a cow (outside the known sleeping sickness foci), was resistant to human plasma in the modified blood incubation infectivity test, providing further evidence of spread of trypanosomes potentially infective to man (Abebe, Dagnachew and Assefa, 1983).

### **Non-Tsetse-transmitted Trypanosomiasis in Cattle**

Stephen (1986) stated that the subject of mechanical, or non cyclical transmission of trypanosomes to domestic animals in Africa and elsewhere, is of more than academic importance. In areas where *Glossina* is eradicated, cases of the disease usually still exist, but there are also regions where tsetse flies are thought not to exist, and yet when an infected animal is introduced, serious outbreaks occur amongst indigenous animals. For reasons not well understood such outbreaks in tsetse-free areas, are more frequently caused by *T. vivax* than *T. congolense*.

Although some of the reports of mechanical transmission are open to doubt because tsetse may be present at very low densities, or the species found locally may

be a crepuscular or even nocturnal feeder and escape observation, Hoare (1947) reported that in addition to tsetse flies, which are the true intermediate hosts of *T. vivax*, these species are readily transmitted mechanically by the other blood sucking *Diptera*, especially Tabanid flies. Thus, it is well known that the area of distribution of *T. vivax* extends beyond that of *Glossina* in various parts of Africa. In contrast Wells (1972) stated that on the African continent there is no clear evidence of nagana trypanosomes being transmitted in the absence of tsetse. However, there is strong circumstantial evidence for non-tsetse fly transmitted *T. vivax*.

In Ethiopia *T. vivax* is found in the entire country except in the highlands which are above 2500 masl and it is thought that the widespread nature of this parasite is due to adaptation to mechanical transmission by biting flies in areas outside the tsetse fly belt (Dagnatchew, 1982). It is indicated that the acyclical transmission of the disease is effected by means of blood sucking flies which include *Tabanus*, *Haematopota*, *Chrysops*, *Hipobosca*, and *Stomoxys* species. Balfour, di Domizio as cited by Hoare (1972) reported that *T. vivax* is prevalent among cattle in Eritrea in Ethiopia and Kasala in the Sudan which are remote from the area of tsetse distribution. Moreover (Roeder *et al.*, 1984) reported high mortality in cattle which were fattened in a commercial enterprise at Melka Sedi which is situated in the hot semi-arid zone of Ethiopia at an altitude of 900 masl caused by *T. vivax* which provided strong evidence for mechanical transmission. During the investigation a large number of biting flies were present in the vicinity of the cattle pens. In addition it has been noted that *T. vivax* infection has a widespread occurrence in eastern Ethiopia where there is no evidence of tsetse existence.

A study conducted in the Gonder region where there has been no record of tsetse-flies, has shown a prevalence of trypanosomiasis of 4.5% and the only species of trypanosome encountered was *T. vivax* (Hassen, 1988). Similarly acute bovine trypanosomiasis in a tsetse-free zone of Nigeria due to *T. vivax* caused serious losses in a sedentary herd at Dalori Dairy farm, Maiduguri. These animals had no contact

with nomadic cattle, but during an outbreak due to high humidity and a warm climate, there had been high insect activity and a high populations of biting flies was thought to be responsible for the spread of trypanosomiasis outside the tsetse zone (Nawathe *et al.*, 1988).

Ford (1964) commented on the geographical distribution of trypanosome infection in African cattle populations. In Rhodesia he observed that *T. congolense* was the predominant species of trypanosome in cattle populations adjacent to *Glossina* distribution, but as the distance away increased so *T. vivax* become the predominant species and later it was confirmed by MacLennan (1970) that the further the herd is removed from areas infected with tsetse the higher the proportion of infections caused by *T. vivax*".

### **Non-Tsetse-transmitted Trypanosomiasis in Camels**

*T. evansi* was the first trypanosome to be described and identified as the causative agent of mammalian trypanosomiasis and early reports of the trypanosome were published by Evans who associated it with an endemic disease in equines and camels known as "surra". Over the years, following these early reports, more discoveries were made of similar disease caused by trypanosomes indistinguishable from *T. evansi* in diverse mammalian hosts in various parts of the world. Thirty-three different names were ascribed to these trypanosomes, but it is accepted that the only valid name for the parasite of "surra" is *T. evansi* (Hoare, 1972).

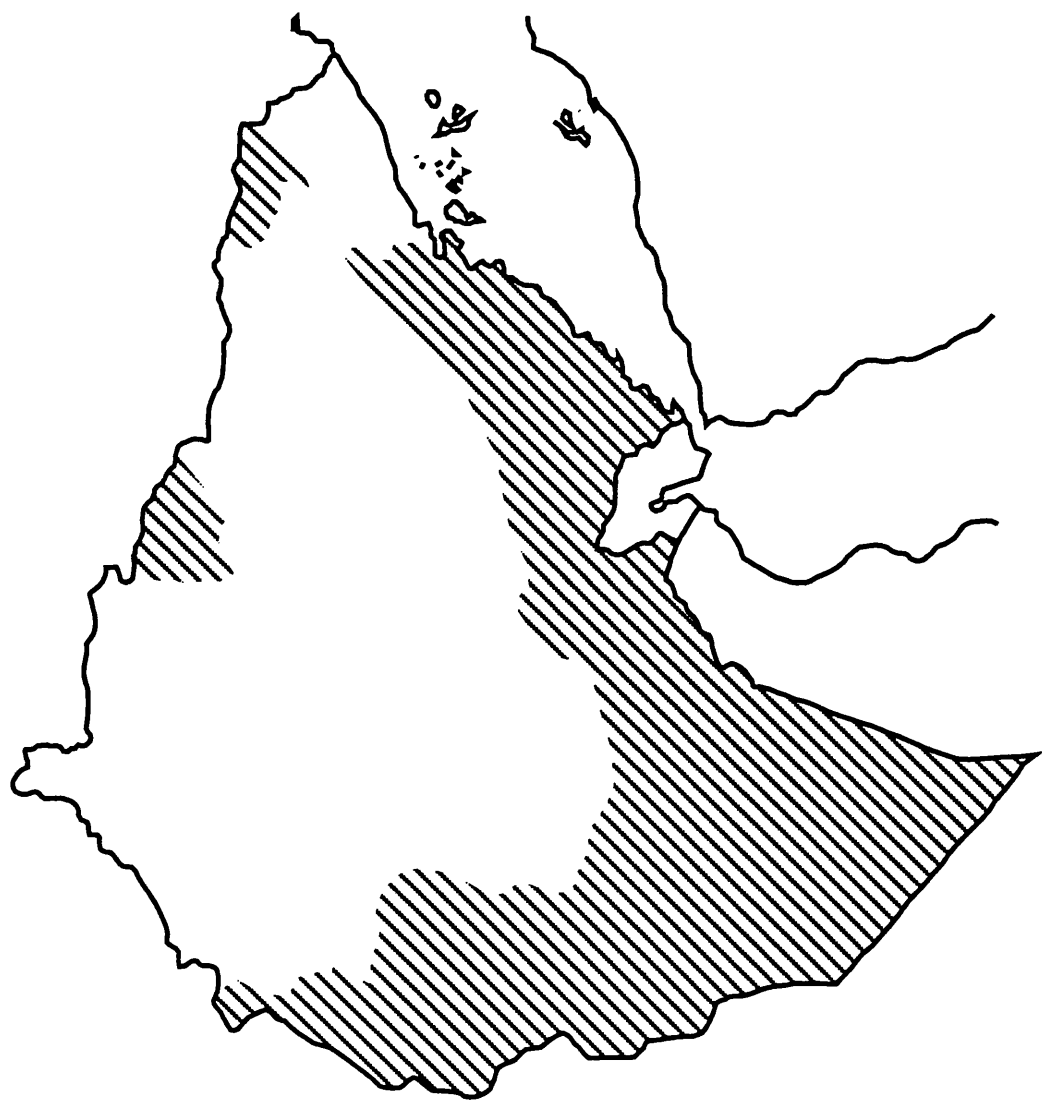
*T. evansi* epidemics tend to involve different animal hosts in different parts of the world. In Indochina horses are mainly affected, followed by camels, bovines and buffaloes; whereas in Soviet Middle Asia the main hosts are camels and to a lesser extent horses. In Africa camels are affected the most. In central and south America, horses are the main hosts followed by cattle. There is a close correlation between seasonal outbreaks of *T. evansi* infections and the increase in Tabanids during tropical rainy season (June to October). However, there is a degree of variation in the

prevalence of different species of Tabanids during the year (Mahmoud and Gray, 1980). According to Yagi and Razig, cited by Mahmoud and Gray (1980), the prevalence of some Tabanid species all year round ensures that transmission of *T. evansi* occurs where reservoir hosts, vectors and susceptible hosts co-exist. This probably explains why sporadic infections are reported during the dry season and outbreaks occur during the rainy season.

In Ethiopia the distribution of *T. evansi* mainly follows the distribution of camels (Langridge, 1976; Dagnachew, 1982). Practically all the camels in Ethiopia are kept by nomadic pastoralists in the arid lowlands of north-eastern, eastern and southern parts of the country where they are essential as a source of food, income and prestigious wealth in the society (Plate 1.5 and 1.6). Despite the fact that camels are very important in the Ethiopian economy few studies on their diseases and husbandry have been carried out. Wosene (1991) reported that in the Ogaden administrative region out of 321 blood samples collected from camels only 21 positive cases of *T. evansi* were demonstrated. Whereas Pegram and Scott (1976) found a high prevalence of *T. evansi* among camels in southern Ethiopia. An investigation carried out in Hararghe and Sidamo administrative regions showed that 14.4% of randomly sampled camels were infected with surra (TCS, 1984). During a drug efficacy trial in Lower Awash (Gewane) a field strain of *T. evansi* was isolated from camels purchased for experimental purposes (Zelleke, Kassa and Abebe 1989).

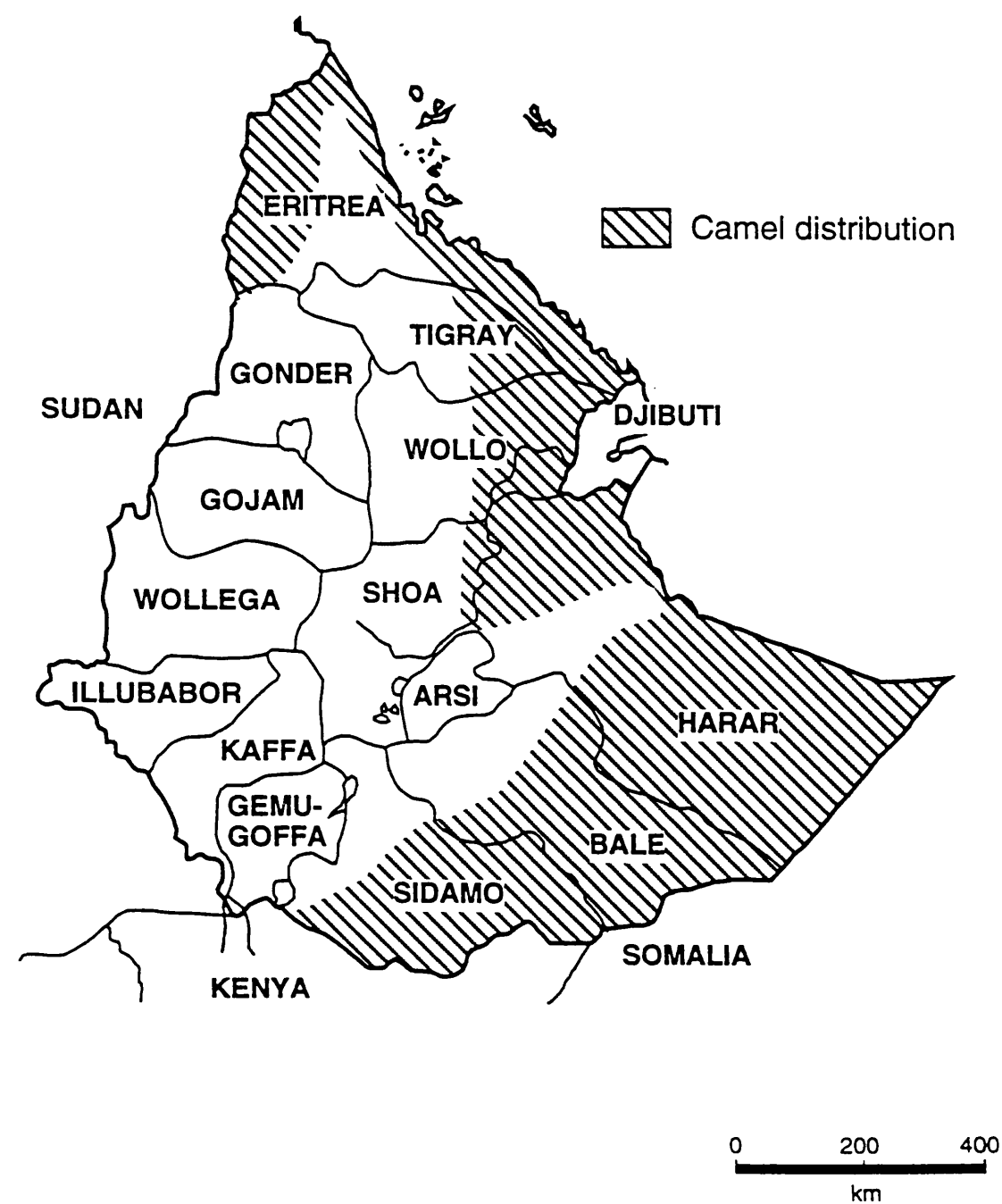
In the Borena administrative region, out of a total of 1100 blood samples examined between November 1989 - March 1990, 237 (22%) were positive. In all cases only *T. evansi* was identified. In addition blood films examined from 58 camels which were suspected of being infected on clinical grounds, 31 (53%) were positive for the same species of trypanosome (Meskelu, 1990). According to Richard (1979) a survey carried out in the Borena region in January 1974 showed infection levels of 20% in camels on the basis of slide examination and 37% when inoculated into mice. The role of insect bites is recognised by all camel breeders in Borena who fear the

**Plate 1.5** *Trypanosoma evansi* Distribution in Ethiopia.



**Source:** D. Richard, 1979.

**Plate 1.6** The Camel Distribution in Ethiopia.



**Source:** D. Richard, 1979.



rainy season when swarms of biting flies occur.

The clinical entities of the disease in camels of Borena were loss of body condition, emaciation, reduced size of the hump, anaemia and oedema in the dependent part of the body (Meskelu, 1990). The disease may be fatal within months or may last for a few years. Spontaneous recovery is considered rare. The disease is also thought to be an important cause of abortion and mortality in young camels (Hornby, 1952; Stephen, 1970; Mahmoud and Gray, 1980).

### **Non-Tsetse-transmitted Trypanosomiasis in Horses**

Non-tsetse transmitted trypanosomiasis caused by *T.equiperdum* is discussed separately in Chapter 3.

## Chapter 2

### The Socio-economic Impact of Trypanosomiasis in Ethiopia

#### Introduction

Every one familiar with the problem of rural development in Africa south of the Sahara is aware that animal trypanosomiasis constitutes a major constraint. Although the disease is primarily related to the prevalence of tsetse-flies, large areas also exist where trypanosomiasis may be a source of disease prevalence even in the absence of cyclical transmission by *Glossina* (Mortelmans, 1984). However, tsetse-borne trypanosomiasis has been widely recognised as one of the biggest obstacles to increased livestock productivity in Africa.

In Ethiopia the predominant farming system in the highlands is mixed livestock and crop productions with livestock playing a vital role in agricultural activities. The provision of draught animals is particularly crucial since crop production is fully dependent up on the use of male adult cattle for draught power. The highland people maintain cattle herds to supply these draught animals (FLDP, 1989). The presence of tsetse-flies in many provinces and the disease, trypanosomiasis, which they transmit have been responsible for large areas of the country being left undeveloped. The impact of tsetse-borne trypanosomiasis on development programmes in rural Ethiopia is becoming more and more important since the avoidance of tsetse-infested areas by the peasantry in general and livestock owners in particular has been common practice to avoid the risk of trypanosomiasis. A land suitability study carried out in areas of low population density in south-western, southern and western Ethiopia revealed that the tsetse-infested lowlands in the western part of the country have the best potential for expanded agriculture, provided that the tsetse/trypanosomiasis problem can be overcome (FAO, 1987). In contrast the eco-system in the highland areas of Ethiopia is becoming fragile because it is over-populated, and this has resulted in land

degradation. Furthermore, part of the northern highlands are subject to recurrent droughts which cause great hardship to man and his livestock.

### **Livestock Production Systems and the Role of Livestock in Ethiopia**

Although there are few commercial farms and ranches in Ethiopia the 1989/1990 census of livestock shows that about 23 million head of cattle, 9.5 million sheep, 6 million goats, and 3 million equines are owned by small private holdings and this data excludes nomadic holdings (C.S.A., 1990). Livestock usage in Ethiopia is very diversified but the most important reasons for keeping livestock include draught power, milk production, meat production, hides and skins for export and for local industries, cash income, manure and often prestige (FAO, 1988, FLDP, 1989).

The social and economic aspects of small-holder production in Ethiopia have not been adequately investigated and the role of livestock in the farming system varies significantly from area to area. However, in general the role of cattle is primarily to provide draught power and manure for tillage as inputs for crop production (FAO, 1988, Kriesel and Lemma, 1989).

Ethiopia has the largest draught animal population in sub-Saharan Africa with about 6 million draught oxen and 5 million equines in the highlands. Over 90% of the farmers in the highlands use animal traction in food crop production. Animal traction helps to cultivate larger areas more quickly. In the highlands there is a positive association between the number of oxen and the area cultivated. (Table 2.1). Shortages of oxen results in delayed seed bed preparation and planting. Adequately available oxen power also enables farmers in the Ethiopian highlands to grow a more profitable mixture of crops. A study in the highlands showed that farmers with more oxen allotted more land to the production of cereals which have a high market value. Since cereal crops (teff and wheat) require more cultivation and more timely seed bed preparation than pulses, farmers with more oxen allotted more land to cereals. In contrast the proportion of land allotted to pulses, crops that need relatively little

**Table 2.1** Effects of oxen ownership on area and cropping pattern at Debre Zeit

Oxen ownership	Crop area/farm (ha)	Area to cereals(%)	Area to pulses (%)
None	1.2	54	46
One	1.9	44	56
Two	2.7	67	33
Three or more	3.6	92	8

*Source Getachew et al., 1993*

cultivation and are planted late in the rainy season, are higher among farmers that have a shortage of oxen ( Getachew *et al.*, 1993 ). The use of livestock as draught power in tropical Africa is not traditional other than in the highlands of Ethiopia which are outside the tsetse distribution. Work oxen are widely used for ploughing, traction and other purposes in the highlands whereas this tradition is precluded in lowland areas ( Jordan, 1986).

In addition to keeping cattle for draught power cows usually provide milk for the cattle-owning household and sometime for local sale. In the range lands this is one of the staples of the pastoralist diet. Although sales for beef are secondary to the maintenance of cattle for milk, most males are surplus and are regularly sold for slaughter or fattening in the commercial sector to raise cash ( Kreisel and Lemma, 1989, FLDP, 1989 ).

Although the presence of livestock disease in Ethiopia limits the livestock and livestock byproduct trade in the international market these products are the second most important export commodity after coffee. Live animals are exported to Middle East countries and different parts of Africa while chilled and frozen meats are acceptable in other countries (FLDP, 1989). Investment of crop income into cattle ownership leads to capital growth as the herd grows through reproduction.

All of the above outputs are in principle amenable to quantification and economic valuation. Inputs include fodder through consumption of straw or stubble from cultivated land, grazing land and hay and the cost of veterinary care (Barrett, 1991, Kriesel and Lemma, 1989).

### **Quantifying the Economic Losses due to Trypanosomiasis**

Evidence accumulated in the past shows that tsetse-flies in Ethiopia have not reached their bioclimatic limit. This has been shown in the Rift Valley and in the Omo and Abai river systems (Balis and Bergeon, 1970; Langridge, 1976; Fuller, 1978; Chater, 1982; FAO, 1987 & 1989). Tsetse distribution is progressing in an upstream direction within the river valleys which cut into the central highland plateau

and towards higher altitudes. With the gradual saturation of non-infested highlands by livestock owners, pressure on tsetse-infested resources has increased steadily, as has the intensity of contact between the fly and animals. Thus livestock is being pushed into contact with the tsetse at lower altitudes. The distribution of the fly is also dynamic and farming areas at higher altitudes are being progressively invaded by tsetse. This phenomenon can be understood better when taking into account the specific topography of the western and south-western parts of the country where, long, often steep-sided tsetse-infested valleys penetrate into the highland plateau. It is well understood that in epidemiological terms, the more widespread savannah species of tsetse (*G. m. submorsitans* and *G. pallidipes*) are of greater importance than the riverine species. For instance the advance of *G. m. submorsitans* in the Bedelle area has resulted in an exponential increase in the annual trypanosomiasis incidence in livestock even at 1900 - 2000 masl. Disease transmission has increased and most of the cattle become re-infested approximately within a month of treatment. The prevalence of the disease correlates closely with the abundance of tsetse (Slingenbergh, 1992).

Trypanosomiasis can affect livestock in many ways and is a disease of considerable economic importance. The cattle industry is particularly seriously affected whilst other species including horses, donkeys, mules, camels and small ruminants are also at risk. Australian Agricultural and Consulting Management (AACM) as cited by Slingenbergh (1992) made clear that the geographical distribution of most domestic animals is strongly influenced by tsetse. Equines particularly horses are very rare below 1700 metres. Cattle densities below 1700 m when expressed as the number of tropical livestock units (TLU) per km<sup>2</sup> are generally less than 5-10 TLU/km<sup>2</sup> with vast lowlands areas devoid of cattle. In the 1700 to 2000 m altitude range cattle are much more numerous with 10 to 35 TLU/km<sup>2</sup>, whilst sheep and goats are evenly distributed over the different altitude ranges. In the highlands above 2000 m cattle densities usually exceed 50 TLU/km<sup>2</sup>.

In general the number of cattle estimated to be at risk from trypanosomiasis in western and south-western Ethiopia varies from one observer to another since nationwide surveys have not been made, however, there is no doubt that millions of cattle are exposed to this disease. Dagnachew (1982) estimated that over 10 million head of cattle are at risk from trypanosomiasis in Ethiopia. The National Tsetse and Trypanosomiasis Investigation and Control Centre (NTTICC) in 1986 confirmed this figure and stated that if other susceptible species of livestock also had been included the number could have been by far greater. FAO as cited by Slingenbergh (1992) made clear that although the Ethiopian highlands above 2000 m are generally free of tsetse-flies in areas below the 2000 m contour some 6 million cattle are exposed to tsetse-flies. Recently Tadelle as cited by Nuru (1993) stated that about 5.5 million cattle are at risk from trypanosomiasis of which 20,000 are dying every year. Slingenbergh (1992) has reported that between 1700 - 2000 m, there are 2-2.4 million cattle distributed and he estimated on average that these animals have at least 2-3 infections per year and over 80 - 90% of all trypanosomiasis infection occur in these uplands, out of which 10% of the infections result in death. Putt (1990) estimated that about 4 million head of cattle graze between 1700 - 2000 m on the plateau at sometime during the year and are consequently at risk of contracting the disease. He estimated in the Limu-Shay area which is in the Upper Didessa Valley that 20% of the infected animals die in the absence of treatment but he appreciated that mortality rates in infected and untreated susceptible cattle are often considerably higher than this estimation. The Fourth Livestock Development Project (FLDP) a tsetse control review and project identification mission in 1989, recognised that the advance of tsetse-flies in the Upper Didessa Valley during recent years has resulted in losses of livestock (equines, cattle and small ruminants) of up to 80 - 90 per cent. The loss of large numbers of oxen for draught power ( the community of Chello alone lost over 500 draught oxen) has had major socio-economic consequences because of the resulting decrease in cultivated land.

The socio-economic data that has been collected from two neighbouring sites during 1989 and 1990 in the Upper Didessa Valley where one was in a tsetse controlled area (Limu-Shay) and the other one outside the tsetse controlled area (Gale) has shown a disease prevalence of 4% and 24% respectively. In the former, 95% of the herd had survived up to the end of the year of study the remaining 5% of the herd was accounted for by death due to different factors or by sale or slaughter for consumption. In the area without tsetse control only 44 % of the cattle had survived to the end of the year, of the remaining 56% many had died, mainly due to trypanosomiasis although other offtake was responsible for part of the total not accounted for at the end of the year. The survey also showed decreases in herd value over the year by 3%, which amounted to 61,170.7 Ethiopian (Eth.) Birr for Limu-Shay and 43% valued at 424,332.5 Eth. Birr for Gale. The loss in Gale was attributed to tsetse-transmitted trypanosomiasis. Similarly Nuru in 1993 indicated that the overall prevalence rate of trypanosomiasis in Gale and Limu-Say was 24% and 4% respectively. The trypanosome species encountered were *T. congolense* and *T. vivax*. In both areas *T. congolense* appeared to be predominant and he stated that due to this high trypanosome incidence in the Gale area the human population had retreated from the area. There was a loss of all livestock types; a lowering of nutritional status and farm incomes in the area and it was very difficult to produce cattle for meat, milk, skins and draught power. However, some of the farmers purchase oxen seasonally for cultivation and resell the animals before they become infected with the disease. Therefore, oxen are the only cattle found in the area. Jemal *et al.* (1993) also made a similar observation that in Gale draught power is the primary reason for keeping cattle.

Animal traction is an appropriate, affordable and sustainable technology which is traditionally acceptable. Draught animals, notably cattle, provide small-holder farmers with vital power for cultivation. They complement both hand labour and motor power. They are mainly used for ploughing, harrowing and threshing. Even when mixed farming is practised, the important contributions of cattle of work and



manure are not their only functions in most communities. Often more importance is attached to their social significance than as providers of food or income. Trypanosomiasis has been a major cause of the failure to develop a tradition of true mixed farming in the lowlands where cropping is possible and the disease precludes the keeping of any significant numbers of domestic livestock (Jordan, 1986).

The Ministry of Agriculture Resettlement Office as cited by NTTICC (1986) has officially stated that in 1985/1986 in areas such as Metekel in the Gojam and Kishe in the Kaffa administrative regions serious losses occurred among introduced oxen which succumbed to trypanosomiasis in a very short time. Out of 1085 oxen introduced in Metekel 101 died, and 606 were sold. Similarly from 1000 oxen introduced in Kishe 425 died and the rest of the animals were moved out of the area. Investigations carried out in Gambella, Kelem, Kaffa and Mizan for two years around resettlement schemes and other trypanosomiasis problem areas to acquire more information on the situation and to take remedial measures to reduce the effects of the disease have shown that unless measures are taken to abate the strong challenge from trypanosomiasis the resident population as well as new settlers are being exposed to serious problems which jeopardise the enormous effort made to establish the settlements. It is also of grave concern that areas of recent tsetse advance include old villages. This is particularly true in the Upper Didessa and Wamma Valleys where *G. m. submorsitans*, as mentioned earlier, is invading new areas. Peasants in these localities have been forced to abandon their villages to move to nearby highland areas, after suffering heavy losses of their cattle. Many of the farmers were left without work oxen and have turned to hand tilling. A good example is the case of the Gojeb peasants producers co-operatives which were formed mainly by the peasants who left their villages in the Upper Didessa Valley to be settled on the nearest high ground in the Bedelle district of Buno province, Illubabor region. They bought about 30 oxen through contribution from each member and started using them for cultivation. In three years they lost 24 of their oxen and were forced to use hand

hoes again. Similar situations are suspected to be common in different areas where tsetse advance has taken place (NTTICC, 1986).

### **Nature of Losses Caused by Trypanosomiasis**

The losses of livestock due to trypanosomiasis depend mainly on the type of production system, however, the major concern in Ethiopia is in rural communities where mixed agriculture is the livelihood. The losses imposed by tsetse/trypanosomiasis can be clearly divided into two forms, direct and indirect. The direct losses include those resulting from mortality, morbidity, infertility and the costs of implementing and running trypanosomiasis control operations. Indirect losses are due to the risk of the disease and include the exclusion of ruminant livestock production from tsetse-infested areas, reduced livestock production levels due to restricted grazing, and reduced crop production due to exclusion or limitation of draught power ( ILRAD, 1993; Shaw, 1987 ).

As already noted susceptible livestock are at continuous risk wherever tsetse are present, the severity of the problem varying within very wide limits and involving the vector , the breed of animal and its physiological condition and the strain of trypanosome. In practical terms the presence of tsetse completely precludes the maintenance of livestock in large areas of Africa. In these regions domestic livestock may die after an acute or chronic illness, or if non-fatal, trypanosomiasis may cause severe losses in production due to poor growth, weight loss, low milk yield, reduced capacity to work, infertility and abortion. As trypanosomes are not necessarily detectable in affected animals the disease is not always correctly diagnosed and as a result its importance may not be properly assessed. The clinical manifestations in the animal also vary and can be exceedingly acute, when it is usually rapidly fatal. However frequently it is more chronic in type giving rise to a debilitating illness also often fatal if not treated. Losses therefore, take the form of mortality and morbidity

It is widely recognised that in Ethiopia millions of domestic animals are at risk from trypanosomiasis. In the following section the extent of these losses in cattle, in sheep and goats, in equines and in camels will be assessed.

### Losses in Cattle

Almost all cattle owned by smallholders are dependant on pasture and most are of the East African zebu type. The herd composition as surveyed by FAO (1988) can be estimated to be 48% male (61.7% ox, 23.5% bull and 14.8% male calves) and 52% female (61.2% cow 19.4% heifer and 19.4% female calves).

The annual reproduction rate for cattle in Chelo averaged 42% (based on mature females). Mortality rates in 1986/1987 were 63% in calves and 39% in adult cattle, while offtake was 11% (FAO, 1988). The annual reproduction rate in Limu-Shay which is in the tsetse controlled area was 64.3% whereas in the case of Gale it was only 44.4%, the difference between the two is 19.9 which appeared to be mainly due to trypanosomiasis (Yemenu, 1993). The mortality rate in Gale was 26% (Jemal *et al.*, 1993). The average milk yield per lactation per cow was 582 litres and the average price was 0.5 Birr/litre (Yemenu, 1993 and Putt, 1990), whereas Habtemariam in 1983 estimated that in south-western Ethiopia a cow can give about 4 pound of milk per day over a lactation period of 8 months with an estimated price of 0.6 Birr/pound (0.45/litre) of milk. Draught power which is a crucial part of agricultural practice in Ethiopia is also seriously affected. According to Putt (1990) a trypanosomiasis-infected ox takes 30 days after treatment to recover from an infection and cannot work during this period. The actual period of the year when an animal is working is 120 days for ploughing, cultivation and threshing. The price of hire of draught oxen is about 2 Birr per animal per day.

The loss of meat that results from trypanosomiasis has not been studied in Ethiopia but studies in other countries show that in experimentally infected cattle, weight losses of over 30% are commonly reported (Wellde *et al.*, 1974). In Nigeria losses of 8-21% were reported in Boran cows infected with *T. congolense* (Llewelyn

*et al.*, 1988). Finelle in 1983 estimated that the average productivity benefit in Africa was 12.5kg per head per year when the trypanosomiasis problem was controlled.

**Assumptions**

Based on the above reviews and personal knowledge of most of the above mentioned areas in Ethiopia, the following assumptions have been made in order to estimate the losses in cattle through mortality and morbidity. It should be made clear that the figures given are conservative estimations.

- a) The cattle population at risk of trypanosomiasis in areas below 2000 metres is estimated to be 6 million which is in agreement with FAO (1991b) as cited by Slingenbergh ,1992.
- b) Based on the current epidemiological situation which was discussed in the previous chapter out of the total number of cattle at risk at least 40% are estimated to take 2-3 infections per annum out of which 15% may die. The remaining, 85%, may suffer from the chronic form of the disease which is believed to cause serious production losses. The price of cattle is taken as suggested by Yemenu (1993) to be as follows:

Female Animals	Price in Birr	Male Animals	Price in Birr
Calf	110	Calf	110
Heifer	278	Bull	285
Cow	390	Oxen	610

- c) The crucial part played by cattle in agricultural practices in Ethiopia is draught power because crop production is heavily dependant on traction power. As suggested by Putt (1990) the duration of time for an infected ox to recover fully with proper treatment takes 30 days/year. It is also assumed that most infected oxen receive treatment and hire prices of oxen are Eth.Birr 15 per day.

- d) Milk loss is also a very important part of the socio-economic impact which affects the nutrition level of the community and their source of income from the sales of milk , cheese and butter. As mentioned earlier the predominant cattle are zebu which normally give an average of 2 litres of milk/day, in addition to that taken by the calf for the lactation period of 8 months. Milk losses of 30% are estimated in tsetse-infested areas due to trypanosomiasis. Currently the price of milk and its by-products ( cheese and butter) are very expensive in rural areas, particularly in places where keeping livestock is difficult. Therefore, in this study the price of milk is estimated 1.20 Birr/litre.
- e) Infertility estimated to be 15% in cows.
- f) Although there is no study carried out locally on meat losses due to trypanosomiasis it is believed that the situation is similar to other African countries. For this reason the loss of meat from infected animals is estimated to be 12.5 kg per year. The average price estimated to be 3 Birr/kg.

## **I Direct Losses Due to Trypanosomiasis**

### **a) Losses in Cattle**

#### **1) Losses through mortality**

Number of cattle at risk	6,000,000
Number of cattle infected annually 40% of the population	i.e., 2,400,000
Number of animals dying every year.	15% i.e., 360,000

The herd composition of these 360,000 cattle is taken to be the same as the composition of the national herd which is 48% (172,800) male (61.7% ox, 23.5% bull calf and 14.8% calf) and 52% (187200) female (61.2% cow, 19.4% heifer and 19.4% calf).

<b>Males</b>	<b>No. of Animals</b>	<b>Unit price in Birr</b>	<b>Total price in Birr</b>
Ox	106618	610	65,036,980
Bull calf	40608	285	11,573,280
Calf	25574	110	<u>2,813,140</u>
		Sub total	<u>79,423,400</u>

<b>Female</b>	<b>No. of Animals</b>	<b>Unit price in Birr</b>	<b>Total price in Birr</b>
Cow	114566	390	44,680,740
Heifer	36317	278	10,096,126
Calf	36317	110	<u>3,994,870</u>
		Sub total	<u>58,771,736</u>
		<b>Total</b>	<u>138,195,136</u> =====

The estimated losses of male and female cattle due to mortality is estimated to be 138,195,136 Birr per annum.

## 2) Losses through morbidity

### a) Draught power

Total animal infected = 2,040,000 of which 48% are the male

$$2040000 \times 48/100 = 979,200$$

The number of oxen assumed to be 61.7% of the male group i.e.,

$$979200 \times 61.7/100 = 604166$$

Therefore, the estimated losses from draught power is,

$$604166 \times 30 \times 15 \text{ Birr} = 271,874,700 \text{ Birr}$$

The annual estimated draught power losses due to trypanosomiasis is 271,874,700 Birr

### b) Milk

The number of female cattle exposed to trypanosomiasis infection is 52% of the total infected herd The number of cows is 61% of the female population out of

30% may occur due to the effects of trypanosomiasis. If milk production is taken as 2 litres per cow per day and the price as 1.20 Birr/litre.

Therefore,

$$2,040,000 \times 52/100 = 1,060,800$$

$$1,060,800 \times 61/100 = 647,088 \text{ of which } 70\% \text{ gives effective calving}$$

$$647,088 \times 70/100 = 452,962 \text{ cows}$$

$$452,962 \times 2 \times 30 \times 8 = 217,412,760 \text{ litres of milk out of which } 30\% \text{ loss is estimated.}$$

$$217,412,760 \times 30/100 = 65,226,528 \text{ litres of milk}$$

$$65,226,528 \times 1.20 \text{ Birr} = 78,271,834 \text{ Birr}$$

The milk losses due to trypanosomiasis is estimated to be 78,271,834 Birr.

### c) Infertility

This problem is common in Ethiopia as in most tsetse-infested African countries and the losses due to infertility are high. According to Putt (1990) losses due to infertility between tsetse controlled and uncontrolled areas in western Ethiopia showed a difference of 10%. However in this study taking into account abortion cases and failure to conceive it is estimated that 15% of cows in the tsetse-infested areas may face infertility problems which account for calf and related production losses.

Therefore, the total number of infected cows 647,088 of which 15% are believed to be infertile.

$647,088 \times 15/100 = 97,063$  cows, if the problem of trypanosomiasis was not there, these animals would produce a calf at least once in two years. The price of the calf taken as 110 Birr.

$$97,063 \times 110/2 = 5,378,465 \text{ Birr per year.}$$

The estimated losses from fertility is 5,378,465 Birr per year

d) Meat loss

In chronic infections of trypanosomiasis in cattle emaciation and retarded growth are common features which reduce the amount of meat production. The average meat production loss is estimated at 12.5 kg/animal/year (5% of the TLU) which is in agreement with Finelle (1983). The price is taken as 3 Birr/kg of meat.

Therefore, in this study the loss of meat is calculated for 85% of the infected population (the survivors) which takes the number of infection per year to :

$$\begin{aligned} 2,400,000 \times 85/100 &= 2,040,000 \text{ cattle} \\ 2040000 \times 12.5\text{kg} &= 25,500,000\text{kg of meat} \\ 25,500,000 \times 3 \text{ Birr} &= 76,500,000 \text{ Birr} \end{aligned}$$

The estimated total meat production loss due to trypanosomiasis is 76,500,000 Birr.

**Total Direct Losses of Cattle Production**

Mortality	138,195,136
Draught power loss	271,874,700
Milk production loss	78,271,834
Infertility .	5,338,465
Meat production loss	76,500,000
.....	
Total	<u>570,180,135</u> Birr per annum

The total annual losses through mortality and morbidity of Ethiopian cattle is estimated to be 570,180,135 Birr.

b) Losses in Sheep and Goats

Most of the studies on the epidemiology of sheep and goat trypanosomiasis in the tsetse-infested areas of Africa have revealed that the disease can be of considerable importance. The study carried out by Opsana and Onyeka in Nigeria at



Fashola, ILCA station showed that of 44 West African dwarf sheep and 38 goats monitored for the disease 18.1% and 34.3% respectively were positive for trypanosomiasis. In sheep, the infections were *T. vivax* 75%, *T. congolense* 12.5% and *T. brucei* 12.5% and in the goats, *T. vivax* 84.6% and *T. congolense* 15.4% were identified from positive cases. Zewart *et al.* in 1973 conducted a serological survey (using the indirect florescent antibody test) in the Kiboko area of Kenya and found that 39% of the sheep and 44% of the goats possessed antibodies against *T. congolense*, *T. vivax* and *T. brucei*. Although Mutayoba *et al.* (1985) suggested that East African goats in tsetse-endemic areas of East Africa can withstand chronic infection with *T. congolense* to varying degrees, their experiment also showed that when the goats were exposed to needle challenge infection with *T. congolense*, they developed acute, sub acute and chronic infection which resulted in 30-70% mortality and rapid weight loss.

In general it is clear that trypanosomiasis in sheep and goats in tsetse-infested areas of Africa has been given little attention compared to other domestic livestock. However, recent studies indicate that sheep and goats are important hosts of African trypanosomiasis, and their infection can lead to serious economic losses (Llewelyn *et al.* 1987; Kanayari *et al.* 1983; Griffin and Allonby, 1979). The impact of trypanosomiasis in sheep and goats as in cattle causes production losses through mortality and morbidity.

In Ethiopia sheep account for about 18% of the total livestock population and goats take fourth place with their numbers accounting for 14% (NAE, 1988). Seventy five per cent of the sheep and 23% of the goats are considered to be located in areas above 1500 masl. Sheep and goats are a source of household food for peasant and pastoralist communities supply, notably for special occasions, and income generators through regular sale. Wool and milk production from sheep and goats are not important activities in Ethiopia (FLDP, 1989).

### **Assumptions of Losses made for Sheep and Goats**

Currently from the national population 50% of sheep and 80% of goats are assumed to live in areas below 2000 m out of which 10% of the sheep and goats may develop a number of trypanosome infections per year. From the infected population at least 6% and 4% respectively may die. The remaining 94% of the sheep and 96% of the goats are believed to develop a chronic infection which causes production losses. In rural areas of the western and south western Ethiopia the price of sheep and goats varies depending on body weight but if one takes an average weight of 25kg for sheep and goats the price range can be estimated at 100-140 and 80-90 Birr respectively. From this the average price is taken as 120 Birr for sheep and 85 Birr for goats. During chronic infections the farmers may loss 25 Birr from each sheep and 20 Birr from each goat per year due to weight loss and retarded growth. In this study the only losses to be considered are losses through mortality and losses through morbidity particularly weight loss.

#### **1) Losses through mortality in sheep**

$24,000,000 \times 50/100 = 12,000,000$  sheep live in areas below 2000 m out of which 10% will take a number of infections per year.

$12,000,000 \times 10/100 = 1,200,000$  sheep estimated to be infected out of which 6% die .

$1,200,000 \times 6/100 = 72,000$  sheep die every year and each sheep is worth 120 Birr

$72,000 \times 120 = 8,640,000$  Birr is the accounted loss

The estimated loss from sheep mortality is 8,640,000 Birr per year

#### **2) Losses through morbidity in sheep**

$1,200,000 \times 94/100 = 1,128,000$  sheep are estimated to have a chronic infection with losses at a rate of 25 Birr per animal.

$$1,128,000 \times 25 = 28,200,000 \text{ Birr}$$

The estimated losses from trypanosomiasis through morbidity in sheep is estimated to be 28,200,000 Birr.

The total estimated losses through mortality and morbidity in sheep is 36,840,000 Birr per year.

### 3) Losses through mortality in goats

The total number of goats below 2000 m is,

$$18,000,000 \times 80/100 = 14,400,000 \text{ goats}$$

of which 10% will take a number of infections per year.

$$14,400,000 \times 10/100 = 1,440,000 \text{ goats believed to be infected}$$

of which 4% may die.

Therefore,

$$1,440,000 \times 4/100 = 57,600 \text{ goats die every year and if}$$

each goat is estimated to be worth 85 Birr.

$$57,600 \times 85 \text{ Birr} = 4,896,000 \text{ Birr per year.}$$

The estimated loss through mortality in goats is 4,896,000 Birr

### 4) Losses through morbidity in goats

$$1,440,000 \times 96/100 = 1,382,400 \text{ goats develop a chronic infection.}$$

Each goat is estimated to lose weight equivalent to 20 Birr.

$$1,382,000 \times 20 \text{ Birr} = 27,648,000 \text{ Birr.}$$

The estimated losses through morbidity in goats is estimated to be 27,648,000 Birr per year. The total estimated loss through mortality and morbidity is 32,544,000 Birr per year.

In the sheep and goats therefore the total estimated losses through mortality and morbidity as described above is 69,384,000

### c) Losses in Equines

Equines are mainly use as transport and pack animals in Ethiopia. They can improve the efficiency of farm management and the utilisation of manure and residues. They also improve crop marketing and sustain trade with a range of social and economic benefits. The distribution of horses, donkeys and mules varies at different altitudes. Horses are normally abundant in the highland areas above 2000 m and few occur at lower altitudes, whereas mules and donkeys are more numerous below 2000 m. They are all susceptible to tsetse-transmitted trypanosomiasis particularly *T. brucei* infection which affects them very seriously. In tsetse-infested areas their numbers are highly variable. On the other hand a field survey conducted in Bale and Arsi regions found that thousands of horses are probably dying every year from dourine. Although the disease shows seasonal fluctuations most of the infected animals die or remain unproductive for any type of work unless they receive proper treatment.

For this reason it is estimated that 3% of the equine population in Ethiopia may get a number of infections every year either from tsetse-transmitted trypanosomiasis or dourine out of which at least 40% may die because of lack of proper treatment and the remaining (60%) suffering from chronic infection. Although the value is different for each subspecies (horse, mule donkey) for this study the average price of 180 Birr/animal is taken. For those remaining sick with chronic infections it is estimated that the loss of income through infertility and debilitation and can be estimated to be 70 Birr per animal.

#### 1) Losses through mortality in equines.

$7,000,000 \times 3/100 = 210,000$  equines become infected every year of which 40% may die.

$$210,000 \times 40/100 = 84,000 \text{ equines die.}$$

The average price is estimated at 180 Birr/ animal. Therefore, the loss of equines through mortality is estimated to be

$$84,000 \times 180 \text{ Birr} = 15,120,000 \text{ Birr per year}$$

## 2) Losses through morbidity in equines.

The estimated number of infected equines is 210,000 of which 60% may develop chronic infection . Therefore,

$210,000 \times 60/100 = 126,000$  equines are estimated to be suffering from chronic infection. The loss expected per animal is 70 Birr. That is,

$$126,000 \times 70 \text{ Birr} = 8,820,000 \text{ Birr per year}$$

The total estimated losses in equines through mortality and morbidity is 23,940,000 Birr per year.

## d) Losses in camels

One tends to think of camels as fated to endless wandering through the desert. Indeed, they made invasions possible, contributed to trading exchanges, and are the favourite animal of nomadic populations. They are often kept in arid areas where few other stock can survive. In less barren pastures they can eat plants left uneaten by cattle, thus allowing a more comprehensive utilisation of the land (Richard, 1979).

As stated earlier in Ethiopia there are about one million camels. They are distributed in the plains surrounding the central massif in the North, East, and South (Dagachew, 1982). They are not found in the Western part of the country and this is believed to be due in part to the high risk of trypanosome infections. The Northern and Eastern plains rarely receive more than 300 millimetres of rain per year. These are sub-desert areas with few pastures. More than two-thirds of the total Ethiopian camel population is found in the South-eastern and Southern plains since they offer them a less severe environment (Richard, 1979).

A study conducted in the Borena administrative region has shown that camels are the most reliable asset and essential for the life and subsistence economy of the pastoralists. In areas where there is persistent water scarcity and recurrent occurrences of drought they play a vital role in supporting the community in that area

by providing direct food (milk, meat) and transport services as pack animals (Richard, 1979; Meskelu, 1990).

However, for the time being, the above advantages are not fully exploited because of disease and nutritional limitations. In camels surra is the predominant disease. The agent responsible is *T. evansi* which is transmitted mechanically by biting flies. The disease has a chronic character. Only after the disease has progressed for a month, or longer, does the animal begin to show signs of illness, normally recognised by progressive weakness, tendency to tire, reluctance to rise, stumbling and loss of condition. The hair coat becomes rough and staring. Body condition deteriorates progressively. A decrease in the size of the hump is particularly noticeable. Oedema is usually seen on the ventral aspects of the body, particularly on either side of the sternal pad, in the sheath in the male, and in the udder in the female. These swellings may become infected and suppurate especially at pressure sites. Pregnant animals may abort, and even full-term calves are often weak and thin and frequently die ( Stephen, 1986).

The consequences of this infection are very detrimental to livestock owners in camel-keeping areas of Ethiopia (Pegram and Scott, 1976, Richard, 1979, Zeleke, Kassa and Abebe,1989). Similarly, camel trypanosomiasis is found to be a serious problem in neighbouring Kenya (Olaho and Wilson, 1983) and in Somalia (Dirie, 1989). As cited by Stephen (1986) Rutter stated mortality in camels due to surra, is around 3% even when in most instances there has probably been some chemotherapeutic intervention.

Although the epidemiology of surra has been reviewed by several scientists no detailed assessment of the economic impact of the disease has been attempted. For this reason the following assumptions and particularly the prices are based on personal communication and information from local markets.

### **Assumptions made for the Estimated Losses in Camels**

Camel trypanosomiasis (surra) has a seasonal character coinciding with the distribution of mechanical vectors. It is assumed that about 15% of the camel population in Ethiopia may become infected each year of which about 5% may die due to lack of proper treatment. The remaining 95% may develop chronic infections that contribute to production losses, infertility and abortion. Depending on the sex and age the average price of a camel in the rangelands of Ethiopia varies from 400 to 600 Birr and in this study the average of 500 Birr is used.

The weight loss is estimated to be 18kg/ camel and the price of meat is estimated to be 1 Birr/kg since the market demand for camel meat is lower in most parts of the country except in the rangelands.

Milk production is not included because it is for home consumption only.

The losses in camels in this study include losses through mortality and morbidity, where only meat loss is considered.

#### **1) Losses through mortality of camels**

$$1,000,000 \times 15/100 = 150,000 \text{ camels may become infected annually.}$$

$$150,000 \times 5/100 = 7,500 \text{ camels die every year.}$$

$$7,500 \times 500 \text{ Birr} = 3,750,000 \text{ Birr}$$

The estimated annual loss from camel mortality is estimated to be 3,750,000 Birr per year.

#### **1) Losses through morbidity**

$$150,000 \times 95/100 = 142,500$$

$$142,500 \times 18\text{kg} = 2,565,000 \text{ kg of meat.}$$

$$2,565,000 \times 1 \text{ Birr} = 2,565,000 \text{ Birr.}$$

The estimated annual loss through morbidity of camels is estimated to be 2,565,000 Birr.

The total loss through mortality and morbidity of camels is estimated to be 6,315,000

The estimated annual loss through morbidity of camels is estimated to be 2,565,000 Birr.

The total loss through mortality and morbidity of camels is estimated to be 6,315,000 Birr per year

**Total Estimated Direct Livestock Production Losses from Trypanosomiasis in Ethiopia**

Losses from cattle	570,180,135
Losses from sheep and goats	69,384,000
Losses from equines	23,940,000
Losses from camels	<u>6,315,000</u>
Total	<u><b>669,819,135</b></u> Birr per year

**II Control costs**

In general uncontrolled trypanosomiasis has devastating effects on crop production through reductions in the supply of draught power. Where the tsetse and trypanosomiasis problem is severe and no intervention takes place, livestock production becomes impossible and farmers are either reduced to cultivation by hand or have to abandon the area. Such economic losses are very difficult to quantify and value, particularly in respect of how the situation is likely to change over time (Barrett, 1992).

Efforts have been and are being made by the Ethiopian government to control trypanosomiasis by the use of trypanocidal drugs. In addition to this, since 1986 a tsetse control operation using odour baited traps and targets has been underway against *G. m. submorsitans* and *G. tachinoides* in the upper Didessa Valley of western Ethiopia. Both control techniques require substantial amounts of foreign exchange.

**Chemotherapy and Chemoprophylaxis**



veterinary (insufficient treatment intervals, under-dosing) and biological (development of chemoresistance). Nevertheless, animal trypanosomiasis has largely been controlled in Ethiopia by curative and prophylactic drug treatments since the mid 1960's. For this reason different type of trypanocidal drugs have been imported from different parts of the world to be used for the control of animal trypanosomiasis.

A study carried out on the importation and domestic distribution of trypanocidal drugs in Ethiopia indicates that from 1978 to 1982 five different type of trypanocidal drugs were imported. These were: diminazine aceturate, isometamidium chloride, homidium bromide, quinapyramine sulphate and suramin which amounted to a total of 9,672,575 doses. In addition the cost of drug administration was calculated at the rate of US\$ 0.5 per dose (TCS, 1983). In the years from 1983 to 1987 over 13.9 million doses of trypanocides were imported and distributed. Despite these numbers, the quantities appeared to be insufficient for a sustained country-wide control of the disease (FLDP, 1989). From 1987 to 1992 over 3,985,000 doses of trypanocides were imported and distributed (AISCO, 1992). In 1993 alone 2.1 million doses of trypanocidal drug were imported by the African Development Bank (ADB) and the World Bank (WB). This data does not include contributions from Non-Governmental Organisations (NGO) and illegal imports which are regarded as considerable (AISCO, 1993).

As far as domestic distribution is concerned diminazine aceturate is the most widely used drug followed by isometamidium chloride, homidium bromide, quinapyramine sulphate and suramin. It appears that the largest quantity of diminazine aceturate was distributed in the Shoa administrative region, which was followed by Kaffa, Sidamo, Wollega, Gamo Gofa and Gojam. But this may be misleading as many customers come from different regions and get the drug from the main drug store in Addis Ababa which is in the Shoa region. In the case of isometamidium chloride the largest quantity was distributed to Gamo Gofa region, followed by Wollega, Shoa, Sidamo, Kaffa, Illubabor and Gojam. However the bulk

of trypanocidal drugs were utilised in the tsetse-infested regions, namely Shoa, Kaffa, Wollega, Gamo Gofa, Sidamo, Illubabor and Gojam (TCS, 1983).

Since the 1980's the recommendations for drug usage by the Veterinary Services Department in Ethiopia has been based on the level of risk of trypanosomiasis, namely high risk, medium risk and low risk. In low risk areas where trypanosomiasis incidence is low, treatments as required with diminazine aceturate is recommended. In medium risk areas two treatments with isometamidium chloride at intervals of four months are recommended followed by diminazine aceturate treatments at one month intervals for four months. In high risk areas four treatments with isometamidium chloride at intervals of two months, followed by four diminazine aceturate treatments at one month intervals for four months are recommended (MOA, 1984).

The current cost of trypanocidal drugs in the country still seem affordable by the peasant communities. Barrett (1992) referring to NTTICC and FAO technical staff indicated that the estimated annual cost of an effective trypanocidal drug could range from US\$ 2-10 per animal depend on the level of the tsetse challenge and he agreed that this is a similar order of magnitude to known costs in other countries. In the Angar-Gutin settlement one of the high risk areas with abundant *G. m. submorsitans* challenge strategic use of trypanocidal drugs was initiated in the 1970s. At the beginning only the curative, diminazine aceturate was used every 28 days. Later the prophylactic, isometamidium chloride was introduced and eventually a regime of alternating the two drugs was established to avoid the development of drug resistance (Bourn and Scott, 1978). This study clearly demonstrated that with careful management, good veterinary supervision and the judicious use of drugs, a very acceptable level of trypanosomiasis control can be achieved in areas of high tsetse challenge using available methods, essentially because of the animals, ability to develop an adequate level of non-sterile immunity with the assistance of chemotherapy (Holmes and Scott, 1982).

Although efforts are being made by the Veterinary Services Department there are difficulties with trypanocidal drug distribution and usage. These include problems with illegal traders, poor controls on drug quality and unsupervised treatment when incorrect diagnosis and underdosing occurs reaching to a failure to maintain regular treatments in medium and high risk areas.

### **Drug resistance**

As has been reported from many parts of Africa (Dolan *et al.*, 1992; Mbwambo *et al.*, 1988 and Williamson 1976) drug resistance can limit the efficacy of chemotherapeutic and chemoprophylactic control programmes. In Ethiopia the first record of drug resistance followed a study conducted in the Arjo and Angar-Gutin settlement areas by Scott and Pegram in 1974 when they isolated a strain of *T. congolense* resistant to homidium bromide in the local zebu cattle.

In the Kaffa administrative region (Ghibe, Abelti and Abilu Ghibe) a Berenil resistant strain of *T. congolense* was isolated (Dagnachew, 1985). Later in west Abaya failure of Berenil therapy to eliminate early infections with a strain of *T. brucei* was recorded (Abebe, 1987). More recently in the Ghibe valley of south-west Ethiopia a strain *T. congolense* was isolated which was resistant to diminazine aceturate at a dose of 7.0mg/kg body weight, isometamidium chloride at a dose of 0.5mg/kg body weight and homidium chloride at a dose of 1mg/kg body weight (Coja *et al.*, 1993; ILCA, 1990). Other tsetse-infested areas may have similar problems.

### **Tsetse control operations**

The tsetse control programme was initially started in 1986 through FAO project TCP/ETH/4523 with the aim of adapting a low cost vector control technique to Ethiopian conditions using odour baited insecticide impregnated traps and targets, suitable for use by village communities on a self-help basis. It was established with an initial budget of US\$ 248,000. Since the trial gave positive results, field testing of the technique began in May 1987 with a further US\$ 160,000 provided under

TCP/ETH/6765 (E) to control tsetse over an area of some 150 km<sup>2</sup>. Results were again successful, but highlighted the need for a long-term project to consolidate the achievements gained through the previous two TCP projects, to expand the area treated, to develop the capability of NTTICC staff and to provide facilities to undertake large-scale future programmes. In June 1988, ETH/88/Uo1 was initiated by UNDP with a total budget of some US\$ 435,500 to fulfil NTTICC's needs over the following 18 months with the objective of eradicating tsetse-fly from an area of 700 km<sup>2</sup> in western Ethiopia and to strengthen the capacity and experience of the NTTICC so that by the end of the project it would be capable of implementing a large scale and sustained national tsetse and trypanosomiasis control campaign without external assistance (FLDP, 1986; FAO, 1989). Funding of the latter project and its technical activities terminated in November 1990 and bridging finance was secured by the World Bank for two years with a budget of US\$ 399,415 donor contribution and 265,500 Eth. Birr government contribution. The main priority of this project is to reduce the costs of the eradication technology. Accordingly an expansion of the existing operations in an area of between 300-400 km<sup>2</sup> is proposed. This area will encompass the Gale settlement scheme (Putt, 1990). This project is financed by the World Bank through FLDP and its activities are still in progress.

### **Research and investigation**

As the problems posed by trypanosomiasis became apparent the Ethiopian government established a National Tsetse and Trypanosomiasis Unit in 1976 at Debre Zeit which took full responsibility for the investigation of the disease and vector. In 1982/1983 the unit had 9 technical and 27 auxiliary staff and a recurrent budget of 223,262 Birr out of which 125,472 Birr was for salaries and 97,790 Birr for petty cash (T.C.S, 1983). The capital budget for the same year is believed to have been far higher than the above mentioned figures but details are not available. Similarly by 1983/1984 a total budget of 181,396.04 Birr was utilised, and the source of finance was the Ministry of Agriculture (T.C.S, 1984). By 1989 the number of technical staff

reached 27 with a further expansion to 69 over the next 3-5 years has been planned assuming that the institute would take on an operational role (FLDP, 1989).

## **Summary of Trypanosomiasis Control and Investigation Costs in Ethiopia**

### **a) Annual cost of drug prophylaxis and treatment**

As described previously from 1978 to 1993 the amount of trypanocidal drugs obtained through official channels was 29,657,575 doses with an average amount of trypanocidal drugs used per year of 1,853,908 doses. Although the amount of drug available is small compared with the demand, treatments given by the veterinary clinics are affordable for peasants because all the overhead costs are normally covered by government subsidy. For this reason trypanocidal drugs currently available in government veterinary clinics cost only 4 Birr/dose. If it was not subsidised the cost would be raised at least by two fold, and from this the annual estimated cost to Ethiopia of trypanocidal drugs can be calculated.

$$1,853,598 \times 8 \text{ Birr} = 14,828,784 \text{ Birr per annum}$$

### **b) Annual cost of tsetse control**

Although the ongoing programme is on a small scale relative to the total area infested by different species of tsetse-flies in the western and south-western part of the country the achievements are very encouraging. Since 1986 the total area cleared of tsetse in the Upper Didessa Valley has been nearly 800km<sup>2</sup>. Initially the programme was designed to test whether target technology was effective or not in the Ethiopian situation particularly on the two important species of tsetse, *G. m. submorsitans* and *G. tachnoides*. Then step by step the programme expanded, community participation increased and this has helped to minimise the costs of labour without affecting the peasants' normal activities.

For this reason the actual cost of tsetse control in Ethiopia at this stage is about 1.4 million Birr per year. However to clear whole infested areas would

For this reason the actual cost of tsetse control in Ethiopia at this stage is about 1.4 million Birr per year. However to clear whole infested areas would require substantial resources and manpower. For instance experience in Zimbabwe has shown that target operations cost from US\$ 120 to US\$ 320 per km<sup>2</sup> and these costs do not include monitoring, buildings or administrative costs (Putt,1990). This suggests that to eradicate tsetse flies from the entire belt in Ethiopia may take, if successful, at least 25 to 30 years. The cost actually depends on the type of technology used. A combination of techniques with integrated development scheme seems preferable for effective results. This may cost at least US\$ 85- 90 million with minimum contingencies of 20% during the project life.

#### **c) Annual costs for diagnosis and research**

NTTICC activities are greater and the number of technical staff has increased steadily since the investigation and research programmes started in the early 1980's. The annual budget of this institution has also greatly increased because the government is seriously concerned about the impact of trypanosomiasis on such a large area of the country. The expenditure of the institute is believed to be in the range of 3-3.2 million Birr/year. The total country expenditure for the control of and research against the disease and its vector is estimated to be about 19,228,785 Birr annually.

In summary the conservative estimate of the annual direct cost of trypanosomiasis in Ethiopia is approximately 700million Birr out of which 97% is accounted for by livestock and livestock production losses and 3% is accounted for by control, research and investigation. In comparison the country's capital investment for education and health since 1984 is in the range of 70-80 million Birr per annum (PMGE, 1984).

### III Indirect Losses due to Trypanosomiasis

More important than direct losses caused by the disease, but even more difficult to express in economic terms are the indirect losses to rural communities by inhibiting the development of Ethiopia's natural resources. Where the human population is low, and land is plentiful trypanosomiasis control is most simply accomplished by avoiding areas of dense infestation of *Glossina* (Jordan, 1986). Although the adoption of systems which includes the avoidance of high risk areas or using them at times of the year when the risk is low, may reduce the losses from trypanosomiasis, the land resource is not utilised to its fullest potential. These losses in the potential productivity of infested land resources in economic terms is far more important than the direct mortality and morbidity losses due to trypanosomiasis (Putt, 1982). By avoiding grazing or farm land subject to tsetse infestation, farmers or herders may contribute to land pressure in other areas outside the tsetse-infested zone thus reducing their productivity. This is commonly the case with nomadic livestock and with high human population densities, where farm sizes are reduced and output per farmer is often lowered (Shaw, 1987).

In arable farming systems family groups are less efficient at producing a marketable surplus if they do not possess effective work oxen for ploughing. It has been stated already that the possession of work oxen increases the production of family groups six fold but it has been the usual experience that if cattle-keeping for draught purposes is encouraged in tsetse endemic areas, losses mainly attributable to trypanosomiasis are frequent. The risks are considerable and, generally speaking, it would be wrong to encourage a farmer to get into debt for the purpose of purchasing work oxen in tsetse-infested areas. Tractors are not the complete answer to this problem since, compared with animals, they cannot use the freely available natural resources, do not provide by-products (milk and manure); cannot be eaten; have no

In Ethiopia the largest area of potentially arable land is found in the South and West at altitudes under 2000 m which is tsetse-infested. Most of the population therefore live in an overcrowded manner in the highlands (T.C.S., 1984). For example, Ethiopia with an estimated population of 45.5 million (ONCCP, 1986) spread over 1,223,000km<sup>2</sup> of land, has an average population density of 37 persons/km<sup>2</sup>. However as shown in table 2.2 the average conceals the significant variations in density among the different regions of the country. Six of the 30 regions have less than 10 persons /km<sup>2</sup> while another six have more than 100, and the remaining divisions are in-between. It is also estimated that 37% of the Ethiopian population lives above 2200 m, 45% between 1500-2200 m and the remaining 18% below 1500 m (Kloos and Adugna as cited by Arowolo, 1990). This indicates that much of the land in the potentially arable lowlands is unpopulated and under-utilised.



**Table 2.2** Ethiopia's population distribution.

Region	Population size	Area in km <sup>2</sup>	Population density (persons/km <sup>2</sup> )
Eritrea	2,853,125	98,407	29.0
Tigray	2,581,258	49,725	51.6
Assab	316,955	74,825	4.3
Dire Dawa	404,786	30,743	13.1
Ogaden	795,796	182,748	4.3
Northern gonder	1,640,698	60,758	26.9
Southern Gonder	1,539,999	17,078	90.6
Northern Wello	1,430,115	30,939	46.0
Southern Wello	2,431,693	22,204	110.5
Eastern Gojam	1,418,615	13,936	101.4
Western Gojam	1,848,965	16,836	108.8
Metekel	265,483	29,457	9.1
Assosa	435,507	23,228	18.9
Wellega	2,188,289	43,288	50.1
Northern Shoa	2,145,583	30,855	67.5
Addis Ababa	2,312,723	5,103	429.0
Western Shoa	2,762,575	21,563	105.1
Southern Shoa	2,762,575	16,775	162.5
Eastern Shoa	821,970	12,649	65.2
Western Hararge	1,203,719	33,189	36.5
Eastern Hararge	2,350,319	90,620	26.1
Arssi	1,807,902	23,866	78.6
Bale	880,001	67,327	13.1
Gambela	128,687	26,066	4.9
Illubabor	2,321,835	33,428	70.3
Kaffa	802,371	39,954	20.6
Northern Omo	2,469,090	30,465	82.3
Southern Omo	184,484	22,412	8.4
Sidamo	2,502,920	19,734	131.7
Borena	557,228	83,180	6.7
Total	<b>45,486,000</b>	<b>1,223,000</b>	Mean = <b>37</b>

Source: ONCCP, Regional Planning Departement, 1986.

The total size of western and south-western Ethiopia which comprises Wellega, Illubabor, Kaffa, Gamo Gofa, parts of Sidamo and Shoa according to Langridge (1976) amounts to 325,000 km<sup>2</sup> out of which currently about 220,000km<sup>2</sup> is infested by different species of tsetse (Slingenbergh, 1992). The distribution of these areas in altitude range is,

below 1700 m	135,000km <sup>2</sup>	42%
1700-2000 m	84,5000km <sup>2</sup>	26%
above 2000	105,500km <sup>2</sup>	32%

It is known that cattle distribution is strongly influenced by the altitude. below

1700 metres	5-10%
1700 to 2000 m	25-30%
above 2000 m	60-70%

This shows that about 68% of the western and south-western part of the country or 18% of the total area of the country is tsetse-infested. This large area is believed to be increasing due to a new tsetse advances as described by MacLennan (1980).

A detailed assessment of the effect of tsetse on livestock and crop production carried out in Chelo, near the limit of tsetse distribution in the Didessa river system showed that before the fly invasion, average cereal production per household was 1.6 hectare (ha) with the use of 2 oxen. Following invasion by *G. m.submorsitans*, farmers lost 80% of their livestock. By 1986 cereal production declined to 0.7 ha per family, using 1 oxen. The dramatic impact of trypanosomiasis on the farming system has affected each and every household through almost complete losses of all livestock, contraction of crop areas owing to the loss of work oxen and a shift in crops from high-value teff to maize, with production of the latter often based on hand cultivation. There was increased reliance on homestead gardens for food supplies; dramatic lowering of nutritional status and farm income, abandonment of homesteads in many localities and re-invasion of scrub-woodland in the old cultivation areas, thus providing increased land cover suitable for tsetse. The area was no longer self-

sufficient in food production and an increasing number of people migrated to the highland shoulder (Slingenbergh, 1992).

There is no doubt that in other tsetse-infested areas similar livestock and crop production losses occur. As stated by MacLennan (1980) the lack of draught power may cause production losses of over 80% in the estimated tsetse-infested areas of Ethiopia which is about 220,000 km<sup>2</sup>. It is thought that if disease were not a problem 70% of this area might be suitable for mixed agriculture. It is difficult to estimate indirect losses in monetary terms since the development of the tsetse-infested lowland areas requires an integrated development programme. However, as described above it is believed that indirect losses are far higher than direct production losses.

### **Control options and strategies**

Having established that a tsetse/ trypanosomiasis problem exists, the question of which strategy to implement depends on technical feasibility, availability of resources and cost-effectiveness of different approaches. On the technical side the nature of the project area, the type of vegetation, the number of animals or humans to be treated or protected with drugs etc. will be important. The overall level of costs will also be influenced by these factors, hence choices can be made as to whether an all-out attack to eliminate severe losses or a limited attempt at controlling a minor or intractable problem is required. The question of timing is also a crucial part of the choice of strategy (Shaw, 1986).

Control methods can be divided into two main types

1) Trypanosomiasis control

2) Tsetse control

#### **1. Trypanosomiasis control**

##### **a) Trypanocidal Drugs**

The livestock industries in most tsetse-infested countries of Africa have been almost completely dependant on the use of trypanocidal drugs for treatment and

prevention for most of this century. However, only a small number of drugs are available and they are more than 30 years old. Some very well known and valuable drugs have disappeared from the market. The incentive for pharmaceutical industries to invest in research to develop new effective trypanocides is low (Williamson, 1970; Holmes and Scott, 1982; Mortelmans, 1984; Murray and Gray, 1984).

Nevertheless, chemotherapy and chemoprophylaxis are still very valuable and very useful tools to combat and prevent trypanosomiasis provided one is aware of the limitations of their use and provided one is prepared to use them in the context of the entire economic system of animal husbandry in Africa.

#### Advantages

- \* they are cheap and affordable in comparison with other techniques;
- \* they do not have adverse effects on the environment;
- \* they are safe to animals and to humans who might consume the animals.

#### Disadvantages

- \* it is difficult to ensure that trypanocides are applied correctly by the farmers;
- \* the possible development of drug-resistant parasites;
- \* they only kill the parasite and not the vector therefore the area under challenge may expand.

#### **b) Trypanotolerant Livestock**

It has been recognised for many years that certain breeds of cattle are relatively resistant to the pathogenic effects of trypanosome infections. These trypanotolerant breeds have been most clearly identified in West Africa but some recent studies have indicated that some localised breeds of cattle in East Africa including Ethiopia may be trypanotolerant (Mortelmans, 1984; Trail *et al.*, 1985 and ILCA, 1990).

The exploitation of trypanotolerant breeds of cattle offers one of the most important approaches to the control of animal trypanosomiasis (Murray *et al.*, 1983). In Ethiopia, there are several parts of the country where cattle belonging to breeds

which are not classically regarded as trypanotolerant but are successfully maintained in some degree of contact with tsetse (Langridge, 1976).

Gimira province is one of the highest tsetse challenge areas in Ethiopia. In spite of this, humpless cattle of the Sheko or Gimira breed also called Mizan or Goda, are present in sedentary village herds with multiple ownership. According to Alberio and Hailemariam (1982) and Abebe (1992) these short horned, humpless or small humped cattle have the local reputation of being "trypanotolerant". In contrast recently introduced zebu cattle require regular drug therapy to survive in this area.

#### Advantages

- \* have no negative impact on the environment;
- \* does not require specialised scientific or biological attention;
- \* the methodology lies more in the areas of social approach and organisation;
- \* can provide a sustainable method of control.

#### Disadvantages

- \* it is a long term exercise over several decades;
- \* it may not be easy to convince livestock owners to upgrade or change the existing breed;
- \* still requires trypanocidal drugs and tsetse control in high challenge areas;

#### **Tsetse control**

Current methods of tsetse control can be either chemical or non-chemical. Chemical controls include ground spraying, aerial spraying, impregnated traps and targets baited with chemical attractants.

##### **a) Ground spraying :**

This involves the application of a persistent insecticide to fly resting and refuge sites. This is usually done by a large number of small teams of workers carrying pressurised knapsack sprayers filled with insecticide solution which can be applied using vehicle mounted fog generators (Jordan, 1986). The technique was developed after the Second World War, when synthetic organochlorine insecticides first became

commercially available and was carried out in East Africa from the 1950s onwards, initially using DDT (Wilson as cited by Barrett, 1994). Studies conducted in Zimbabwe showed that the annual cost of ground spraying ranges from Z\$300-400 per/km<sup>2</sup> and indirect costs are not included. These are access provision, camp construction and maintenance etc. (Barrett, 1994).

The possible advantages are:

- \* it is an established technique;
- \* it is relatively inexpensive;
- \* it does not require sophisticated technique and equipment;
- \* DDT has low human toxicity.

Disadvantages

- \* the use of DDT is not favoured on environmental grounds;
- \* alternative insecticides make the technique relatively expensive;
- \* requires careful planning and good logistic support;
- \* operations can be carried out only in the dry season, after which there is a potential problem of re-invasion.

**b) Aerial spraying**

The aerial application of insecticides for tsetse control has a long history. Indeed, following the development of organochlorine insecticides in the 1940s, the first large scale tsetse control programme using insecticides involved aerial spraying of some 18,000 km<sup>2</sup> in Zulu land (now Kwazulu, South Africa) between 1945 and 1952 (du Toit, as cited by Barrett, 1994). Apart from cost considerations, the aerial application of persistent insecticides has significant adverse effects on the environment (Jordan, 1986). The cost of aerial spraying has been shown to be Z\$591/km<sup>2</sup> ranging from Z\$457 to Z\$774/km<sup>2</sup> (Barrett, 1994).

The advantages are:

- \* relatively large areas can be treated over a short time;

- \* there is no need for extensive ground-based operations involving large members of people and vehicles, with associated logistical problems.

The disadvantages are:

- \* it is relatively expensive;
- \* requires high technology and equipment;
- \* the technical feasibility is uncertain particularly in more rugged terrain and against some species of tsetse;
- \* the longterm environmental impact remains controversial and activities that include aerial spraying are normally subjected to high environmental monitoring expenses.

**c) Odour baited traps and targets**

The technique involves a combination of visual and olfactory stimuli which cause tsetse-flies to approach and enter or land upon the device which traps or kills them. Since different species of tsetse-fly have different host-seeking behaviour, various designs have been developed in different parts of Africa.

The experience in Zimbabwe shows that the annual cost of materials and chemicals per target is Z\$ 44 and the annual cost for manpower, vehicle and equipment amounts to Z\$ 49 per target which is marginally higher than the materials and chemicals cost. With targets deployed at four per km<sup>2</sup> total direct cost of tsetse control is between Z\$290 and Z\$510 per km<sup>2</sup> per year, with an average cost of Z\$370 per km<sup>2</sup>. The indirect costs are assumed to be similar to those for ground spraying and include access provision, camp construction and maintenance, equipment, clothing and consumables. On this basis, the total cost of tsetse control is between Z\$450 and Z\$830 per km<sup>2</sup> per year of deployment, with Z\$607 as the average cost (Barrett, 1994).

The advantages are:

- \* low environmental impact;
- \* cost competitive;

- \* time frame is not crucial;
- \* the method is simple and can be used by local people;
- \* technically feasible;
- \* it can be used for prevention as well as reclamation;
- \* can be operational throughout the year if access is provided

**Disadvantages:**

- \* substantial losses through theft and damage and is dependant on the level of community participation;
- \* extensive ground access required which is dependant on the terrain of the specific area;
- \* large scale operation requiring substantial numbers of staff and vehicles and good logistics;
- \* effective mainly on savannah species (*G.morsitans* and *G. pallidipes*).

**d) Treatment of Cattle with Insecticides**

This approach involves applying insecticides to cattle, such that tsetse-flies pick up a lethal dose when they alight on the animals to feed. The method became feasible only in the late 1980s, with the development of effective and persistent formulations of synthetic pyrethroids, which can be applied either as a cattle dip, spray wash or pour-on treatment.

The cost of deltamethrin dipping for a herd of 800 cattle on Makwaja ranch in Tanzania was reported as US\$3.50 (832 Tanzanian shillings) per animal per year (Fox as cited by Barrett, 1994). The principal factors determining the cost of tsetse control by treating cattle with deltamethrin are:

- the method of application
- the cattle density
- the time for which the treatment must be implemented
- whether cattle are already being treated regularly with acaricides



The dipping method is much cheaper than the pour-on method and is the preferred technique where there are sufficient animals to justify establishing a dip.

#### Advantages of the technique

- \* cost effective where animals are assembled for veterinary care
- \* the method is simple, it does not require sophisticated equipment
- \* possibilities for gaining additional benefits from the control of other ectoparasites;
- \* it can be integrated with other bait systems of tsetse control;
- \* it does not involve indiscriminate application of insecticides in the ecosystem.

#### Disadvantages

- \* the technique can not be used in areas where livestock are not present;
- \* the technique is not fully developed, with optimum procedures are yet to be defined;
- \* there is significant possibility of acaricide resistance developing in ticks.

#### **c) Sterile Male Release Technique**

Although the sterilisation of males by chemicals or gamma radiation is thoroughly studied and defined, this method will never have any chance of success because of fundamental biological constraints in the production of males (one female tsetse produces only 2-3 offspring's every month) which make it economically inconceivable and unrealistic (Mortelmans, 1984).

#### **Issues that Requires Further Attention**

##### **1. The advance of tsetse to higher altitudes**

Since time immemorial the rural communities of Ethiopia have preferred to settle on the highlands. The main reason being the highland area could provide a favourable climate for man as well as livestock, with the availability of fertile land for

cultivation and pasture free of trypanosomiasis and malaria. However this is no longer the case. The highlands are overpopulated, suffer from periodic droughts and the tsetse challenge has advanced up the escarpment. Studies in the Upper Didessa valley have shown highly significant advances of *G. m. submorsitans* since the end of 1960s. Similar advances have almost certainly occurred elsewhere and are probably in progress by different species of tsetse. This has involved colonisation of new areas in the valley floor and an extension of the upper altitudinal limit of the fly from 1600 m in 1976 to 2000 m in 1988 (FLDP, 1989).

If this move is into unoccupied land then the immediate consequences are minimal but where livestock is present the results have been devastating. Very high mortalities in all classes of mammalian livestock cause evacuation of the affected area or the impoverishment of the people who remain. For this reason the advance of tsetse to higher altitudes of Ethiopia is an alarming situation that requires further attention and intervention.

## **2. The need for balanced land use**

Prior to the 1960s trypanosomiasis had relatively little impact on the economy of Ethiopia. There were pockets of human sleeping sickness in the west and domestic livestock, particularly cattle, could not be kept over extensive areas of the lowlands but there was no need to utilise the tsetse-infested areas and there was little incentive to do much about the disease and Ethiopia had no need to establish an effective tsetse and trypanosomiasis control organisation. However in more recent years the significance of the disease has increased enormously and is still increasing due to a number of factors, affecting the highlands including: the loss of soil fertility, over-population and over-stocking.

Awareness of the environmental situation in Ethiopia has increased since the early 1970s and a number of studies (FAO/UNDP, FAO as cited by NCS, 1990) have shown that the present trends in natural resource use pose serious constraints to future development. Soil degradation is the most immediate environmental problem

facing the country. The loss of soil and the deterioration in the fertility, moisture storage capacity and structure of the remaining soils, all reduce the country's agricultural productivity. In the mid 1980s, 3.7% of the highlands (2 million hectares) had been so seriously eroded that they could not support cultivation, whilst a further 52% had suffered moderate or serious degradation. Almost 75% of the Ethiopian highlands are estimated to need soil conservation measures of one sort or another if they are to support sustained cultivation. Soil erosion is a self-accelerating process. It reduces the country's food production by an estimated 1-2% per annum. Erosion and the decline in the humus content of the soil reduce both soil moisture storage and rainfall infiltration. These changes undermine the ability of crops to withstand drought and so exacerbate variations in crop yields. Soil erosion contributes to the famines in the northern and eastern highlands and to the country's structural food deficit (N.C.S., 1990; NAE, 1988). For this reason rural-rural migrations can be considered an option from areas of dense cultivation with low or falling per capita incomes into underutilised areas. Experience in Nigeria has shown that the settlement of new areas in the 1950s led to the disappearance of some *G. morsitans* belts and reduced the extent of the trypanosomiasis problem without specific control measures being undertaken. Secondly, a definite acceleration in the rate of immigration was observed into those areas where tsetse clearance enhanced the attractiveness of a region already being colonised ( Shaw, 1987 ).

### **3. Need for Integrated Livestock Development in Tsetse-infested Areas**

The conflict between tsetse control and conservation dates from the first decades of this century when tsetse control required the direct destruction of wild animals and natural vegetation in tsetse-infested areas of Africa. With the advent of ground and aerial spraying by insecticides in the 1950's and 1960's environmental concern shifted towards the effect of insecticides on non-target organisms. Ground spraying with persistent insecticides such as DDT is increasingly unacceptable. However, the sequential aerosol technique has now been developed to a point where

the environmental impact of the insecticides used for aerial spraying is acceptable in many situations. Recently developed bait techniques of tsetse control have comparatively little direct environmental impact in most situations ( Barrett, 1994 ).

For several decades the driving force behind tsetse and trypanosomiasis activities in Ethiopia has been the objective of successive governments to resettle peasant farmers from the highlands into the western and south-western lowlands on a large scale, in a planned and orderly manner. Interest in large scale resettlement programmes in Ethiopia goes back to the late 1960's, although the land ownership system prior to 1974 restricted opportunities for projects (Barrett, 1992). Against this background the first tsetse and trypanosomiasis survey undertaken between 1974 to 1976 led to the identification of priority areas where tsetse control should be considered, and donor endorsement of the proposed control operations in support of rural development (Ford *et al.*, 1976; FAO, 1984; AACM as cited by Barrett, 1992). For this reason the land use issue is the most important and should be identified prior to the implementation of any large scale programme. Certainly it requires a multi-disciplinary case study to be undertaken particularly in the tsetse-infested western and south-western parts of the country. At the same time there must be a clear cut government plan as to what purpose specific land is required (Barrett 1994). The government and donors involved with tsetse control, are increasingly concerned that tsetse control might contribute to environmental degradation, through the promotion of inappropriate land use. Economic appraisal of tsetse control requires projecting future land use in tsetse affected areas, and the evaluation of alternative scenarios with and without intervention.

#### **4. Sustainable Tsetse Control**

The economics of tsetse control or eradication depend very substantially on its sustainability. The only sure way of insuring that re-invasion of an eradicated area does not take place is to eradicate an entire fly-belt. The topography of the known tsetse-infested areas of Ethiopia is such that many are protected on three sides by a

natural barrier (high altitude) of the surrounding uplands so that re-invasion of cleared areas can only take place from one direction. If barriers were constructed across the neck of the valleys following their clearance, then the distance involved would not be substantial and it is likely that re-invasion of the cleared areas could be prevented at reasonable cost (Putt, 1990). The other major factor in the economics of the operation would be the rate at which the cleared areas are put to productive use since the present human and livestock populations in many parts of the valleys are too low to produce the rate of development necessary to secure a satisfactory return on the investment made in control/eradication. This is because such development will have to take place through the introduction of human and livestock populations from upland areas. However, since there is intense pressure on land in the highland areas, there is no reason to suppose that substantial migration into the valleys would not take place. The operation must, therefore, be correctly integrated and phased with land use planning and the creation of the associated infrastructures to insure that an orderly rate of development ensues, that an appropriate system of land use is adopted and that due consideration be given to the preservation of local fauna and flora (MacLennan, 1980 and Putt, 1990). In assessing the feasibility of different options, technical and economic aspects are usually of primary consideration. However, social feasibility is also an important factor in tsetse control operations in which local farmers participate. To ensure effective and sustainable participation by farmers a close understanding of their attitudes and objectives is essential (Salmon and Barrett, 1994).

## **Discussion**

In assessing direct losses of livestock and livestock production assumptions were made for different species of livestock and their products on the basis of current prices and production parameters in the country. In addition the experiences of other African countries has also been included. Although the estimations may show slight variations from one area to another and from year to year the estimate is believed to

reflect the minimum prices of livestock and livestock products in most areas of the country.

The cattle herd composition in this study of 52% female and 48% male was based on the farming system development survey conducted in 1988 by FAO which indicated then that more female animals are needed for breeding purposes than males and this is true when we see the market more male animals are available for slaughter and cash. Later FAO as cited by Mukassa-Mugerwa and Tegene (1991) reported that 50% of cattle herds were female and 50% male with a of 70% calving rate. The price assumptions made for milk and meat in this study is in agreement with the study conducted by Goshu *et al.* (1989) who confirmed that the producer's price in Ethiopia is too low to motivate farmers to raise production and indicated that the price of milk at the collecting sites of MSFD and Dairy Development Enterprises (DDE) is Eth. Birr 0.5/litre, the average price of beef in Addis Ababa, Nazret, Dera, Alemaya, Harrer and Jijiga is Eth. Birr 1.65 per kg body weight.

It is clearly indicated that the crop and livestock sub-system in the highlands of Ethiopia are complementary to each other, and thus highly integrated. Livestock have output, input, asset, security and investment functions in the farming system whereas crop production in its turn provides fodder as a crop residues and stubble grazing to livestock. About 40% of the feed intake of livestock in the highlands is from cereal straw (Gryseels as cited by Getachew *et al.*, 1991). In addition livestock are the major source of income in the farming system of the highlands of Ethiopia. This has been clearly shown in a study conducted by ILCA at Debre Berhan from 1980-1982 where cash income from the sale of animal and livestock products contributed on average, 83% to the total farm cash income. Cash income from the sale of animals contributed 53%, 96% of which consisted of cattle and sheep sales. Overall, on farm livestock production, excluding the value of draught power, provide an average of 46% of the value of the total farm output (Goshu *et al.*, 1989).

This study has shown the importance of oxen in cereal production in the highlands of Ethiopia. Even in times of bad years (crop harvest failures) the farmers

sell livestock selectively : small ruminants, young cattle, equines, cows, and, in a final desperate stage, oxen. This selective selling strategy shows the high value of oxen in the farming system. Gryseels *et al.* (1987) indicated that at Debre Berhan, farmers with two oxen produced an average of 63% more grain than farmers with no oxen, and 19% more than farmers with one ox. Whereas in Debre Zeit in two different investigations farmers with two or more oxen produced 81% and 118% respectively, more grain than farmers with one ox and concluded that farmers owning more oxen produce more grain.

This study has shown that in areas between 1700-2000 masl the livestock and crop interaction is heavily affected by the advance of tsetse-flies which have caused heavy losses to domestic livestock. In areas below 1700 m livestock keeping is precluded. Apart from that the tsetse and trypanosomiasis problem in Ethiopia has created unbalanced land utilization. More people are migrating to the highlands, causing overpopulation; over-grazing and a devastating effect on the environment, while leaving the fertile lowlands abandoned as unsuitable for humans and domestic livestock. Earlier studies have also indicated that in Ethiopia the advance of tsetse to higher altitudes has a serious socio-economic impact (MacLennan, 1980; Jordan, 1986; Putt, 1990; Barret, 1992 and Slingenbergh, 1992). Studies conducted in other African countries also indicate that tsetse and trypanosomiasis have been the major constraints to livestock development (Hoare 1972; Putt *et al.*, 1982; Jordan, 1986 and Toure, 1989).

To counteract the problems imposed by the effects of tsetse and trypanosomiasis, different control methods have been tested and implemented and heavy resources have been invested to refine techniques and these are still in progress. Results vary from one technique to another and from one ecological zone to another. In a country like Ethiopia however a combination of techniques may give better results than any one single one. Putt (1990) indicated that based on the material costs alone, target technology might become attractive as an alternative to drug prophylaxis in the medium and high risk areas of Ethiopia where stocking rates are in

excess of 25 animals per km<sup>2</sup> Jordan (1986) suggested in areas of low carrying capacity where trypanosomiasis challenge is also low it is more economic to control the disease by chemotherapy than by tsetse control. After examining the effects of various disease vector control alternatives with the aid of an epidemiological model of trypanosomiasis in Southwest Ethiopia, Habtemariam (1983) indicated that the combined use of vegetation clearing, insecticide application, trypanocidal drug usage and settlement (or resettlement) was the most effective and feasible method of disease control. Similarly in Zimbabwe a considerable progress has been achieved under the Regional Tsetse and Trypanosomiasis control programme for Malawi, Mozambique, Zambia and Zimbabwe using a combination of control techniques (Barrett, 1994).

### **Conclusions**

It is justifiable to conclude that trypanosomiasis and its vectors (tsetse) are the greatest constraints to livestock production in most areas of the western and southwestern parts of Ethiopia. Furthermore the advance of the tsetse-fly to higher altitudes in recent years has caused serious socio-economic consequences, reducing the availability of animal products and, more seriously reducing the number of draught oxen with major losses of cultivated land much of which have reverted to woodland, suitable to tsetse habitat. The disease also inhibits development in extensive areas of the country virtually unoccupied by man.

Although it is premature to conclude that the highlands of Ethiopia are no longer capable of supporting agricultural activities it is evident that they are saturated with humans and livestock and the agricultural productivity of the area is declining due to land degradation. For this reason urgent steps are required for improved and balanced land utilization.

In general the trypanosomiasis problem in Ethiopia is not just an animal health constraint, it also influences land usage and threatens crop production. Therefore



trypanosomiasis/tsetse control is not only important for rural communities but for the whole country.

### **Recommendations**

In view of trypanosomiasis impact on agricultural production in the tsetse-infested areas of Ethiopia, the following recommendations should be considered as a matter of priority:

1. Design strategic and sustainable trypanosomiasis and tsetse control programme using a combination of the different techniques that are currently available, namely, chemoprophylaxis and chemotherapy; trapping of tsetse/pour-ons; tsetse control using insecticide impregnated targets.
2. Design a strategic diagnostic capacity.
3. Establish a nationwide tsetse and trypanosomiasis survey to remap the areas affected and identify the species present in the country.
4. Formulate a policy governing the distribution and usage of trypanocidal drugs.
5. Promote research activities on: epidemiology of trypanosomiasis; tsetse ecology; the effects of tsetse on land use (the socio-economic aspects); the promotion of indigenous trypanotolerant cattle.
6. Current activities on tsetse and trypanosomiasis by different governmental and non-governmental organisations should be under one umbrella organisation and that is the mandated institution NTTICC in order to avoid duplication of activities and allow for the proper utilization of resources for identified priorities.

**NB** In order to effectively carry out the above recommendations, the man-power, logistical and financial capability of NTTICC should be reviewed and strengthened accordingly.

## Section II

### Chapter 3

#### Trypanosoma equiperdum - Literature Review

##### Introduction

*Trypanosoma equiperdum* is a species of trypanosome which causes a serious disease known as dourine in equines. It is the only trypanosome of veterinary importance that does not require an arthropod vector (Brown, Hunter and Luckins, 1990).

Dourine has been known to Arabs and other horsemen of Africa and Asia for centuries before it was described in Europe in the 18th century by Ammon, who observed cases in the Prussian stud in 1796-9. The true nature of dourine was determined only when Rouget (1896) discovered trypanosomes in the blood of an infected Algerian horse and Schneider & Buffard (1900) reproduced the disease in a horse inoculated with these parasites. The causative organism was named *T. equiperdum* by Doflein in 1901 only a few days before Laveran and Mesnil in 1901 proposed to call it *T. rougeti*, while later Blacklock and Yorke in 1913 gave the name *T. equi* to a pleomorphic strain of this parasite (Hoare, 1972).

*Trypanosoma equiperdum* like other trypanosomes has various synonyms depending on the language of the individual describing the condition. For example, "ed Dourine" in Arabic, "Mal de coit" or "Maladie du coit" in French, "Beschalkrankhet" or "Bschalseuche" in German and "Dourine" or "covering disease" in English. Because the clinical signs bear some resemblance to human venereal disease it has sometimes been referred to as "equine syphilis" (Stephen, 1986).

Since the beginning of this century dourine has attracted the attention of several investigators and diagnostic and control strategies have been developed against the disease.

## Distribution

Formerly dourine was enzootic throughout most continents of the world but with the introduction of control measures and reductions in the equine population this disease has disappeared from most developed countries (Hoare, 1972). However, it is still enzootic in South Africa, in north-west Africa (especially in Morocco), in parts of Syria, Iraq, Turkey and the Tashekant region of the Soviet Union (Woo, 1977). The occurrence of dourine in South Africa (Bophuthatswana, Transkei, Lesotho), South West Africa, Botswana, Zimbabwe and Swaziland was recorded from 1954-1975 and 1981-1984 at the veterinary research institute in Onderstepoort using a complement fixation test (Barrowman and vanVuuren, 1976; Williamson and Herr 1986). An epidemiological survey carried out in 1975/1976 in a 1500km<sup>2</sup> zone in the Abruzzi region of Italy and Sicily in which 2841 horses and 1958 asses were examined for *T. equiperdum* antibody by the complement fixation test (CFT) showed the prevalence of infection in the population to be 7.4% (Nobili *et al.*, 1979; Caporale *et al.*, 1980 and Balbo *et al.*, 1980). In China only a few cases have been reported in the last decade and the last confirmed case was isolated from a horse in Beijing in 1979 (Lun *et al.*, 1992, 1993). It was apparently common in India at the beginning of this century, but the last reported case seems to have occurred in 1921 (Gill, 1977).

Dourine was very extensive during times when the horses were important militarily, economically and agriculturally. In most countries of Europe and North America the disease was of great concern to veterinary authorities at the beginning of the 20<sup>th</sup> century. It persisted in the USA in herds of range horses on Indian reservations until 1949. The extent of the incidence today is very difficult to determine because the regions in which it is thought to continue to exist are those in which veterinary services are heavily involved with more important and dramatic animal disease, or where such services do not exist (Stephen, 1986).

In Ethiopia the existence of the disease was recorded in 1984 (FAO-WHO-OIE, 1984) but there are no confirmed results available and no detailed studies have

been carried out either on the disease or on the causative agent in Ethiopia. Although no evidence is available except their morphological similarities Uilenberg (1992) reported that this parasite is thought to have originated from the tropical African parasite *T. brucei* where various species of zebra constitute a large natural equine population in eastern and southern Africa. Whereas Hoare (1972) thought it probable that *T. equiperdum* evolved from an equine strain of *T. evansi*. Nowadays it is generally restricted to tropical and subtropical countries although its recent outbreaks in southern Europe and its presence in North America in the 1920's indicates it's potential to occur wherever horses are bred (Luckins, 1992; Luckins, 1994).

## Morphology

Many workers (Molyneux and Ashford, 1983; Woo, 1977; Hoare, 1972; Watson, 1920) have described how *T. equiperdum* is morphologically indistinguishable from *T. evansi*, which is dependent on mechanical (vector) transmission.

Stephen (1986) indicated that in stained preparations *T. equiperdum* is identical to specimens of *T. evansi* (kinetoplastic forms), *T. brucei*, *T. rhodesiense* and *T. gambiense*. Normally it is monomorphic with only long and slender and/or intermediate forms identified. The presence of stumpy, or even postero-nuclear forms have been described, but these are usually seen in strains adapted to rodents. Another characteristic which *T. equiperdum* shares with *T. evansi* is a tendency to produce dyskinetoplastic strains. The spontaneous transformation of typical strains of *T. equiperdum* into aberrant ones has been observed on several occasions. The first case was reported by Tobie (1951) when he maintained a European strain in rodents in the U S A. In 1949 a substrain suddenly became totally dyskinetoplastic, whereas the parent strain remained normal, with only 0.7 per cent of dyskinetoplastic forms. The other was reported by Milder and Dean as cited by Hoare (1972) when a

laboratory strain, which had been isolated in Brazil from an infected mare in 1958, began to show a high proportion of such forms in 1967 and became totally dyskinetoplastic by 1969.

Reproduction is by longitudinal, binary-fission in various tissue fluids, particularly in the reproductive system and in subcutaneous urticarial plaques (Brown, Hunter and Luckins, 1990). In view of the difficulties in detecting the parasite in equine hosts, morphological studies on this parasite have been carried out exclusively in infected laboratory rodents (Hoare, 1970). In practical terms it is a tissue parasite and bloodstream forms are rarely observed, but such forms are important in the dissemination of the organism to different organs. However, it may be seen in centrifuged aliquots of blood or in aspirated lymph from oedematous fluids, vaginal washings or other exudates (Lingard, 1906; Parkin, 1948). Pleomorphism has been reported occasionally (Molyneux and Ashford, 1983). In size *T. equiperdum* falls within the range of the monomorphic strains of *T. brucei* of between 15.5 - 36µm with the mean length 25.6 - 27.7µm (Woo, 1977). Due to the low number of isolates available, research on *T. equiperdum* has been much lower than for other species of pathogenic trypanosomes. *Trypanosoma equiperdum* shows kinetoplastic DNA homogeneity, and strain differentiation depends on pulsed field gradient (PFG) karyotyping and repetitive sequence patterns (Touratier, 1993).

### **Host range**

Under natural conditions the disease is strictly limited to equines (Stephen; 1986; Brown, Hunter and Luckins, 1990) and horses and donkeys appear to be more susceptible than mules (Schulz, 1935). Attempts to transmit *T. equiperdum* from horses directly into other animals has been found to be difficult but they readily infect mice and rats. Attempts to infect domestic ruminants (cattle, sheep and goats) with *T. equiperdum* produced in laboratory animals leads to an unapparent or low parasitaemia with only slight clinical manifestations of dourine, whilst in pigs the

infection is latent and without any pathological changes (Curasson as cited by Hoare 1972 ; Molyneux and Ashford 1983).

The blood of a horse infected with a rodent adapted strain was highly pathogenic for dogs and all other species of laboratory animals and the transmission became so easy that dogs could be infected regularly by feeding them horse flesh from the animals that had succumbed to the disease (Stephen, 1986).

Rodents studies have shown that there is variation in susceptibility and resistance among different laboratory rodents. However, it is possible to consistently maintain the parasite in deer mice (Packchanian, 1963; Ekejindu *et al.*, 1985; Kemenes and Horvath, 1986).

## **Transmission**

Because of its host specific character *T. equiperdum* is restricted to equines in natural infections. The disease has the distinction of being the only trypanosome to be venereally transmitted as a result of its presence in the seminal fluid and mucus exudates of the male genitals of the stallion and the vaginal mucus of the female (Molyneux and Ashford, 1983). The trypanosome is capable of passing through intact mucous membranes, and no wound or abrasion is needed to gain entry to the tissue. However, there are periods in the course of the disease when the parasite is absent from the genital mucosa and lies dormant in the tissues (Watson, 1920). This was practically demonstrated by Parkin in 1948. The other interesting observation made by Robinson (1948) is that foals may become infected with dourine before sexual maturity and on reaching sexual maturity such foals are capable of transmitting the infection during service. The mucous membranes of the eyes and nose of foals can be contaminated by vaginal discharges and transmission to foals from udder lesions of the mare is also possible (Molyneux & Ashford, 1983). Human carelessness may also responsible for conveying the infection when contaminated utensils are used for grooming the horses or unsterilized instruments for artificial

insemination (Hoare, 1972).

*Trypanosoma equiperdum* transmission is erratic and might be dependent on the variable presence of active trypanosomes on the genital mucous membrane at the time of coitus (Watson, 1920). Supportive evidence was provided by Parkin (1948) who exposed seven mares to service by a CFT positive stallion. Two failed to become infected. Henning as cited by Barrowman (1977) states that the parasites disappear periodically from the urethra of the stallion, and apparently also from the vagina of the mare so that breeding animals are not uniformly infectious during the whole course of the disease. This raises the question of whether *T. equiperdum* should be regarded as an obligate parasite of the genital tract or as an interstitial tissue parasite whose transmission is dependent on its irregular presence on the genital mucosa. Considering transmission in relation to the epidemiology of the disease it is clear that with the seasonal breeding pattern of the horse the infected stallion is likely to be the most significant propagator of the infection. Within a 3-4 month annual breeding period the infected stallion showing no interference with libido, may cover a large number of mares whereas the mare is only sexually active for short periods of this season and will normally cease breeding activity on conception.

The transmission of *T. equiperdum* by inoculation of blood and vaginal washes in horses, mules and donkeys has been successfully demonstrated (Parkin, 1948). Furthermore the ability of *T. equiperdum* to cross the blood-brain barrier was demonstrated in the horse when cerebrospinal fluid with low detectable levels of trypanosomes was removed from a dourine infected mare by lumbosacral puncture and used to infect another animal. The parasite was detected in blood smears of the recipient 13 days after infection and the subsequent parasitaemia and clinical course of the disease followed that of naturally infected horses (Barrowman, 1976).

The incubation period, that is to say the interval between venereal transmission and the appearance of visible signs, is quite variable ranging from 2 weeks to 3 months or more (Watson, 1920; Stephen, 1986). In mild chronic cases it may persist

for 1-2 years and occasionally even up to 5 years. In more severe cases the animal dies after several months. Dourine is commonly a fatal disease, with an average mortality rate of 50 per cent, especially in stallions. Infected animals sometimes recover spontaneously (Hoare, 1972). In experimental infections of horses and donkeys it was observed that the interval between infection and the appearance of antibody in the blood as determined by CFT was 10 - 11 days (Watson, 1920).

### **Epizootology**

Since dourine is a venereal disease transmitted by direct contact between its mammalian hosts, its epizootology is simpler than in the case of insect-borne trypanosomiasis, for it is not necessary to take into consideration such factors as vectors and ecology. The propagation of dourine depends primarily on the conditions favouring the transfer of *T. equiperdum* from one equine host to another, eg. among breeding animals in studs and among those in free-range herds. The incidence of dourine in different countries and at different times has varied considerably. This has usually been determined by human activity, such as the importation of horses from affected areas and the degree of effectiveness of control measures (Hoare, 1972; Luckins, 1994). The incidence of infection may be influenced by climatic conditions, for example, when dourine was prevalent in Canada the infection was dormant in winter and flared up in summer (Watson, 1920). However little information is currently available on the epidemiology and relative importance of *T. equiperdum* and dourine. It may be more widely distributed in Africa and elsewhere than is currently recognised, particularly because of the difficulty in distinguishing between the trypanosome species and clinical signs of surra and dourine and the absence of simple diagnostic tests (Touratier, 1993).



## Clinical Signs

The clinical course of the infection depends on the pathogenicity of the trypanosome, the resistance of the breed, and the physical condition under which the animals are maintained. The disease is typically chronic, but it tends to be somewhat milder and more protracted in temperate zones than in warm climates. How much this is due to differences in the pathogenicity of the trypanosome, susceptibility of the host, nutritional status, physical stress or general mismanagement, is impossible to determine (Hoare, 1972; Stephen, 1986).

In most accounts of dourine the evolution of the disease is divided in to three well defined periods or stages to which certain symptoms are ascribed, thus: (1) primary - with oedema, tumefactions and changes in the genitalia; (2) secondary - with plaques and skin eruptions; and (3) tertiary - with paralysis, anaemia and cachexia. However this can be misleading as often there are a series of attacks which follow so closely that the illness is virtually continuous, or there may be prolonged periods during which there are no signs of the disease. Although all of the signs mentioned under primary, secondary and tertiary periods may appear, the implication that they occur chronological in order is not always the case. They may occur in random order (Watson, 1920).

Parkin (1948) and Stephen (1970) have reported that Canadian and South African strains normally cause relatively mild, chronic and insidious disease while the European strain is much more pathogenic, with the course of infection extending over 8 to 10 years.

Watson (1920) and Theiler (as cited by Hoare, 1970) have indicated that in the vascular form of the disease there is usually a mucous discharge from the vagina, and the tail may be raised away from the vulva, which is swollen and inflamed. The irritation causes frequent urination or attempts to urinate and some animals show an increase in libido. Abortions or the birth of weak foals may be seen. In stallions the initial signs may be so slight that they are not noticed but, a more or less persistent

oedematous swelling can be noted on the prepuce and over the whole of the floor of the abdomen reaching along the chest wall to the brisket (Walker, 1918). In mares vaginal oedematous swellings may indicate continuing infection, and these should be examined for the presence of trypanosomes. In both sexes there is loss of condition and the coat becomes rough and staring. The pyrexia is intermittent or remittent, and is more regular during the early stages of infection. Periods of pyrexia may last for 5-6 days and the temperature may reach 104°F - 105°F (40 - 40.5°C). Intermissions last for 3-4 weeks. Cutaneous plaques and oedematous swellings precede, or occur simultaneously with, pyrexia peaks. As the disease progresses febrile reactions become more erratic and affected animals are often hyperaesthetic and restless. Later locomotor disturbances gradually appear. The general constitution is little affected even in severe pyrexia episodes, and the appetite remains good (Watson, 1920; Luckins, 1994). At the latter stage the nervous form of the disease may appear and constant ocular lesions may occur after periodic ophthalmia. With the involvement of the nervous system paresis will take place e.g. in the case of facial nerves, the lips are twisted, the ears may hang and the eyelids droop. Other motor nerves subsequently become affected. This results in incoordination of the limbs and finally paralysis (Woo, 1977). With progressive anaemia, emaciation and paraplegia the animal becomes exhausted, is unable to rise and suffers a lingering death (Watson, 1920; Stephen, 1986). The mortality rate in mares is lower than that in male animals (Hoare, 1972).

### **Pathology and Pathogenesis**

Lingard and Hutya as cited by Hoare (1972) noted that most of the clinical manifestation of dourine are the result of histotropism of *T. equiperdum* especially for the mucosa of the genital organs and for the cutaneous tissues. The pathological effect has been attributed to the secretion by the parasite of a toxin. During natural infections of *T. equiperdum* in equines, oedema spreads along the ventral abdomen

involving the udder or scrotum and prepuce. In the stallion the scrotum sheath and testicular coverings are thickened and infiltrated, and in some cases the testicles are embedded in a tough mass of new sclerotic tissue in which they are scarcely recognisable (Watson, 1920). The trypanosome may be carried by the bloodstream to other parts of the body and invade the skin causing oedematous plaques 40-60 days after the onset of the symptoms (Hoare, 1972). The urogenital tract is inflamed and hydrothorax, hydropericardium and ascites are often pronounced (Brown, Hunter and Luckins, 1990). The occurrence of the nervous form of the disease, superimposed on the oedematous interstitial form, appears to coincide with the presence of the parasite in the CSF (Barrowman 1976, 1977; Luckins, 1994)

In acute cases vaginitis, metritis and cystitis are common. The mucous membrane of the uterus may be thickened with a patchy appearance owing to the presence of hyperaemic areas and is infiltrated with a clear yellowish fluid. In the majority of cases the ovaries are markedly enlarged and show cystic degeneration (Schulz, 1935). In long-standing cases the external genitalia may be fibrosed and ulceration of the genital mucosa may leave depigmented scars (Brown, Hunter and Luckins, 1990). Radiculitis and pleuritis, involving cellular infiltration and degenerative changes of the spinal ganglia extending along the larger peripheral nerves, notably the sciatic nerve is seen as a primary lesion during natural infection in horses (Barrowman, 1976; 1977). The lymphatic glands are enlarged and congested. In chronic cases almost all the lymph nodes are enlarged and softened. The lumbar and sacral regions of the spinal cord are most affected where perivascular leucocyte infiltration in the grey matter is possible (Woo, 1977). It is thought that when the parasites invade the tissues they cause vasomotor disturbances with exudation of plasma and an inflammatory reaction at the sites of irritation, giving rise to the oedematous swellings and plaques. The toxin elaborated in these lesions is carried away through the blood stream, causing inflammation and degeneration of the peripheral nerves. According to this view, the motor and sensory disturbances in the

later phases of the disease are the direct result of these changes, whilst emaciation of the animals is due to atrophy of the muscles served by the damaged nerves (Formad, 1919). The production by the parasite of a toxin has not been proved, but it may be significant since it has been demonstrated that fluid from the plaques killed trypanosomes *in vitro* (Lingard as cited by Hoare, 1972). Likewise, Watson (1920) believed that the sudden death of infected rodents at the height of parasitaemia was due to the release of toxins in the circulation.

A direct relationship between the immunoglobulins present in the cerebrospinal fluid (CSF) and the serum of infected animals has been shown, but the total protein level of the CSF was only about 1/80<sup>th</sup> that of the serum, and positive C F reactions could only be obtained after the CSF was concentrated to a considerable extent. Barrowman (1976) assumed that the low level of immunoglobulins in CSF may provide an "immunogenically safe" environment for the parasite compared with that in the serum, and that variable antigenic types of the trypanosomes may be different from those in the blood.

In rabbits infected experimentally with *T. equiperdum*, it has been observed that the spleen was about one and a half times larger than normal and firm with prominent lymph follicles. The lymph nodes, particularly those in the head and neck, were moderately large. Swellings at the inoculation site measuring 1.0 to 2.5cm in diameter were seen some days after infection. Skin lesions unrelated to those at the inoculation sites were also seen. The cellular elements of the lymph nodes were usually separated by oedema (Moulton, Coleman and Gee, 1974). In deer mice (*Peromyscus maniculatus gambelii*) infected with *T. equiperdum* lesions were detected in many organs and tissues, particularly in spleen, lymph nodes, kidney, heart and brain. Microscopically, there were diffuse with perivascular infiltrations of mononuclear cells, especially plasma cells and pronounced lymphoreticular hyperplasia of spleen and lymph nodes (Moulton, Coleman and Thompson, 1974). In rats perivascular oedema in the internal organs, particularly liver, and changes in

vascular endothelium have been observed (Menshikov and Akhmed, 1990). Chronic *T. equiperdum* infections of deer mice induce a relatively consistent laboratory animal model of human trypanosomal encephalitis (Stevens and Moulton, 1977).

## Immunity

The nature of immune responses of infected individuals to trypanosome and non-trypanosomal antigens has been the subject of investigation by many workers and the results obtained revealed that responses in laboratory animal models differ in some details from those in livestock. Even in livestock there are differences between domestic and wild species (Nantulya, Musoke and Rurangirwa, 1985).

The immunology of the disease caused by *T. equiperdum* has been studied less than that of other species of trypanosomes. *Trypanosoma equiperdum* may be symptomless in native free-range horses. Horses of different breeds, as well as individual animals, vary in their susceptibility to the disease. It is also known that mules are less sensitive than horses and donkeys. This suggests that in addition to variations in individual immunity, equines may possess some innate immunity, possibly acquired naturally through prolonged contact with the disease (Hoare, 1972).

Foals may display protective immunity if born from infected mares. It was noted by Cantrell (1960) that the foals either reject infection or become healthy carriers. In India a donkey foal born from an infected mare, when eight and half months old, failed to react to inoculated dourine blood containing *T. equiperdum* and was therefore subsequently inoculated a second time with virulent dourine material, with a similar result (Lingard, 1906). This indicates that the animal may have had a considerable degree of immunity conferred on it by the dam.

Since animals that have recovered from the disease do not show sterile immunity the type of immunity in dourine may be regarded as premunition (Hoare, 1972). Moreover as in other species of trypanozoon, *T. equiperdum* displays antigenic variation in the course of infection giving rise to relapse strains. Antibodies

protect the host against reinfection with the homologous strains but not against heterologous ones (Cantrell, 1960).

Early studies indicated that immunity to trypanosome challenge is mediated by antibody responses against the variable surface glycoprotein - (VSG) (Nantulya *et al.*, 1979). Mammals respond to an antigenic stimulus by an increase in immunoglobulins of various types and they appear sequentially with the initial response being synthesis of macroglobulin, IgM. This is gradually replaced by gamma globulin, IgG with the IgM level decreasing slowly. In trypanosome infections the response is frequently modified, and the IgM level may remain high for prolonged periods (Clarkson, 1976; Stephen, 1986).

During the development of *T. equiperdum* infection in the rabbit increases of approximately 7-10 times in IgM and IgG were detected (Ross and Regenmortel, 1977). Capbern (1978) observed increased IgM values of 8-10 fold by the third week of infection which later began to fall. According to Tizard (1987) who cited different workers, in the mouse serum levels of IgM and IgG are increased many fold following infection with *T. rhodesiense*, *T. congolense*, *T. gambiense*, *T. brucei* and *T. equiperdum*. However, some variations in serum IgM related to the fluctuating parasite load have been reported with *T. equiperdum* (Baltz *et al.*, 1981).

In general IgM is a better agglutinating antibody and in the presence of complement is also a better neutralising antibody. However, when IgM and IgG were tested for their ability to passively protect infected animals it was noted that IgG fractions appeared to give greater protection (Seed, 1977; Seed and Hall, 1985). In addition to the humoral factors, local phagocytosis plays a part in the immune reaction to *T. equiperdum*. Watson (1920) described this phenomenon in the oedematous patches and plaques of infected horses where serous fluid in the lesions contains actively multiplying trypanosomes up to the 36th hour, but by the 40th hour they have attached themselves to the phagocytes. At the 44th hour most of them were ingested by macrophages, and two hours later only the nuclei of the parasites are recognisable

within the phagocytes and this process correlated with the gradual reduction in size of the swellings until they are completely absorbed by the end of the 5th day.

The most commonly used methods for induction of immunity have been the establishment of infection followed by treatment with trypanocidal drugs and inoculation of irradiated non-infective trypanosomes (Morrison, Murray and Akol, 1985). Under field conditions the infection and treatment method would require exposure to a great variety of trypanosomes antigenic types (Losos, 1986). Balth *et al.* as cited by Vickerman and Barry (1982) noted that three consecutive intraperitoneal injections of 1 microgram of variable antigen of *T. equiperdum* in physiological saline was sufficient to endow a mouse with protection. The primary reason for the absence of sterile immunity is the ability of trypanosomes to undergo antigenic variation resulting in the expression of antigenically distinct surface coats (Vickerman and Luckins, 1969).

### **Antigenic Variation**

According to Cantrell (1960) antigenic variation in trypanosomes has been known since 1905 when it was described by Frank. It is a process by which the trypanosomes switches from one coat to another for survival (Vickerman and Barry, 1982; Barry and Turner, 1991) frustrating all attempts to immunise the mammalian host against trypanosomes. The process is responsible for the chronic relapsing course of trypanosomiasis and finds expression in the periodical fluctuation of parasitaemia. As it rises the trypanosomes stimulate the production of a specific antibody which destroys most of the parasites in the population. Those that survive the crises undergo antigenic changes which render them insusceptible to the preceding antibody but as they proliferate they elicit the appearance of corresponding new antibody. This process repeats itself at intervals of a few days (Hoare, 1972).

African trypanosomes contain two functional groups of antigens; common (stable) antigens and variable antigens (Vickerman and Barry, 1982). For protection

from nonspecific immunity and escape from specific immunity, each trypanosome is covered by a replaceable surface coat composed of the VSG (Variable Surface Glycoprotein) which specifies the VAT (Variable Antigenic Type) of trypanosomes (Barry and Turner, 1991). The variant antigens produced early in the infection tend to develop in a fairly predictable sequence. As the infection progresses, however, the variant antigens become more random. Trypanosomes grown in tissue culture also show spontaneous antigenic variation demonstrating that variation is not necessarily induced by antibody. By means of electron microscopy it can be shown that the variant antigen forms a thick coat over the surface of the trypanosome. When antigenic change occurs the proteins in the old coat are shed and replaced by an antigenically different proteins. Analysis of the genetics of this process indicates that trypanosomes possess a large number of genes for coat protein and that antigenic variation occurs as a result of random gene rearrangement and selection (Tizard, 1987).

Cross and Baltz *et al.* as cited by Vickerman and Barry (1982) indicated that although far less is known about the organisation of VSG in the surface coat of *T. evansi* and *T. equiperdum* it is similar to that of *T. brucei*. In general bloodstream forms of trypanosomes express a series of different VATs and the number which may be expressed is still unknown for any trypanosome species (Frame, Ross and Luckins, 1990). However, the work of Van der Ploeg as cited by Frame, Ross and Luckins (1990) estimated in *T. brucei* a potential VAT repertoire of a clone greater than 1000. In *T. equiperdum* infected rabbits comparing the results of agglutination tests 25 days post-infection with those at 46 and 54 days indicated that specific agglutinin titres decreased, suggesting that antigenic variation occurred during infection (Moulton, Coleman and Gee, 1974). Later, following experimental infection of rabbits with variant clones of *T. equiperdum* derived from the same strain, 101 serotypes were isolated and the corresponding antisera prepared (Capbern *et al.*, 1977). Furthermore, it was demonstrated that there was a loose order of VSG expression.



Some VSGs are preferentially expressed early in infection, while other VSGs are only observed later in infection. This observation indicates that trypanosomes can "reset" their VSG clock whenever they enter an immunologically naive host but in a manner that does not lock them in to a rigid pattern of VSG expression which could be easily blocked (Roth *et al.*, 1991).

The different VATs appear in a loosely defined order in rabbits and have been arbitrarily divided into early, middle and late groups. Infection of naive rabbits with any VAT invariably results in the appearance of parasites expressing VSG1 in the first relapse population, thus allowing the isolation and analysis of independent trypanosome clones expressing this antigen. The difference between "early" and "late" genes is that the entire coding region of the silent gene is generally transferred to the expression site during infection (Raibaud *et al.*, 1983). According to Longacre *et al.* who is cited by Roth *et al.* (1991) in the case of *T. equiperdum* VSG1 gene, the EcoRI restriction sites located outside the coding region of the gene are always transferred to the expression site when the gene is activated.

Cantrell as cited by Doyle (1977) obtained relapse populations of *T. equiperdum* by challenging rats previously immunised with drug-treated parasites with approximately  $1 \times 10^8$  trypanosomes from populations derived in rats originally infected with a single parasite of the strain. It was concluded that since the new population arising in the challenged rats were antigenically different from the immunizing population they could be composed of mutant types which had occurred with an incidence of  $2 \times 10^6$  during the course of infection of the donor rats. The new relapse populations were shown by cross immunisation experiments to consist of several different antigenic types, some antigenic types being common to the populations recovered from different challenged rats.

In general the pattern of antigenic variation has to be considered at two levels, first at the cellular and molecular level, involving switching between VSG, and between VSG genes and second at the population level, to elucidate how

trypanosomes expressing different VATs interact with the host to determine the abundance of particular VATs in the population. Although antigenic variation provides the basis for the fluctuating pattern of parasitaemias, the overall course of infection is governed by several other parameters (parasite-dependant, antigenic variation and host dependent) that may be further complicated by the pathogenesis of trypanosomiasis (Barry and Turner, 1991).

### **Immunosuppression**

Although the host responds to the many antigenic types of trypanosomes throughout the infection, it shows depressed immune response to antigens unrelated to those of the infecting organism (Vickerman and Barry, 1982). Immunosuppression occurs in various forms of trypanosomiasis of livestock and man. Under field conditions it may be manifested by secondary infection which may mask the underlying trypanosomiasis (Losos, 1986). The outcome of this effect is especially severe rendering the infected hosts susceptible to secondary infections, and reducing antibody titres following bacterial and viral infections. It is considered a major feature of the pathogenesis of the disease.

*Trypanosoma equiperdum* infection rarely appears to be the immediate cause of mortality in horses when the nervous system is not involved. The debilitating nature of the disease, however, appears to render affected animals more susceptible to other pathogens and external and internal parasites to which they succumb. African horsesickness and bronchopneumonia are prominent among these secondary infections as recorded by Walker (1918) and Robinson (1948). In contrast, it appears that when the nervous system is involved the course of the disease is progressive and fatal. The susceptibility of infected horses to secondary infections and other parasites may be the result of reduced resistance in a debilitated animal or the direct immunosuppressive activity of the trypanosome infections (Goodwin *et al.* as cited by Barrowman, 1977). However, the degree of immunosuppression is

pronounced in laboratory rodents than in large animals.

Evidence for immunosuppression in experimental animals came initially from observations on impaired antibody response to sheep red blood cells (SRBC) in *T. brucei* infected mice and rabbits which failed to produce detectable anti-SRBC immunoglobulin (Goodwin, 1970; Goodwin *et al.*, 1972 and Longstaffe *et al.*, 1973). Balth *et al.* as cited by Losos (1986) noted that in mice infected with *T. brucei*, *T. gambiense* and *T. equiperdum* the immune depression was not the result of clonal exhaustion as measured by IgM levels, but closely associated with the presence of living trypanosomes in chronic infections. According to Moulton and Coleman (1977) deer mice infected with virulent trypanosomes had decreased immunological responses to injected sheep red blood cells (SRBC), whereas deer mice given radioattenuated trypanosomes had normal or enhanced immunologic response to the injection of SRBC. Later Oyejide and Moulton (1983) observed deer mice infected intraperitoneally with *T. equiperdum* and the temporal sequence of splenic plaque forming cell (PFC) generation to SRBC each week over a period of 5 weeks and compared it to the response of uninfected controls. The anti-SRBC, PFC response of splenic cells from infected mice was progressively suppressed from week one to week five (range 41.1 to 96.7% suppression).

Deer mice infected with *T. equiperdum* and given influenza Ao WSN virus developed severe bronchitis, bronchiolitis and alveolitis characterised by respiratory epithelial necrosis and dense infiltrations of perivascular and peribronchial spaces by plasma cells compared to trypanosome-free control deer mice. Image analysis showed that cellular infiltration of the respiratory tissues was greater during the course of influenza virus pneumonia in trypanosome infected mice. This was explained by the reduced ability of trypanosome-infected mice to mobilize neutrophils. (Oyejide *et al.*, 1982). Moreover it has been noted that *T. equiperdum* infection significantly depressed serum and pulmonary neutralizing antibody titres to influenza virus compared to control deer mice (Oyejide, Moulton and Wolcott, 1985).

## Chemotherapy and Chemoprophylaxis

Because of the variety of domestic livestock which are susceptible to trypanosome infections and the diversity of trypanosome species which are pathogenic for animals of economic importance, the problems of chemotherapy and chemoprophylaxis are even more complicated and formidable than in human trypanosomiasis (Williamson, 1970). Outside enzootic areas, chemotherapy is relatively simple since the species of the trypanosome determines the type of curative compounds used (Losos, 1986).

In general the treatment of dourine in naturally infected animals is controversial since the results of therapeutic trials are extremely difficult to assess because of the difficulty of determining whether the trypanosomes had been completely destroyed. Although the complement fixation test can provide a useful indication of the results of treatment, extensive breeding trials would be required to assure that the disease is not reoccurring (Stephen, 1986).

However, the trypanosome is sensitive to the same drugs as *T. evansi*. It is well known that most of the early drugs were investigated primarily for their use in human trypanosomiasis and the first really successful drug, which is still in use today, is suramin. It was introduced in 1920. It is active against *Trypanosoma brucei* sub-group, but has little or no action against other groups of trypanosome. It is therefore of more use in human trypanosomiasis than animal trypanosomiasis except in cases of *T. brucei*, *T. evansi*, and *T. equiperdum* infections. It is especially of use in the treatment of horses and camels as the other trypanocidal drugs tend to be toxic in these animals. For the treatment of *T. equiperdum* infections suramin and quinapyramine are the drugs of choice. Berenil must be used with care as the horse is relatively susceptible to Berenil toxicity (Anon, 1986).

Suramin (Antipol- ICI; Naganol - Bayer): is used as a curative and prophylactic agent of 1 to 4 months duration against *T. brucei*, *T. evansi*, and *T.*

*equiperdum* in horses, donkeys and camels at 10mg/kg<sup>-1</sup> intravenously for two to three treatments at weekly intervals.

Quinapyramin sulphate (Antrycide sulfate): has been widely used as a curative agent against *T. evansi* and *T. equiperdum* in horses and camels at 3 - 5 mg/kg body weight (Losos, 1986).

Diminazine aceturate (Berenil): is the only member of the diamidine group and the commonest drug currently used against tsetse-transmitted species. It qualifies as a wide-spectrum trypanocide. It is effective at 5 - 7mg/kg levels against *T. brucei* (Losos, 1986). In the USSR in experimental and natural infections of dourine in equines it was possible to treat with one dose of Berenil (Azedine) 5mg/kg body weight (Sabansheive, 1984).

In the laboratory rodent-adapted *Trypanosoma equiperdum* has been used quite extensively as a test organism for therapeutic studies. When the four compounds (suramin, tryparsamide, stilbamidine and surfen) were tested against *T. equiperdum* in Webster Swiss mice, it was found that the drug susceptibility of all the lines was essentially the same. They were all sensitive to suramin, stilbamidine and tryparsamide, but the akinetoplastic sub strain was somewhat more sensitive to the bis-quinaldines (sufren, e.t.c.) than either the parent strain or the pearce- Brown strain (Goble *et al.*, 1959).

In female albino mice inoculated intraperitoneally with  $1 \times 10^6$  viable trypanosomes (*T. equiperdum*), and then given the antibiotics phlomycin (8mg/kg) profiromycin (10mg/kg) and mitomycin (4mg/kg) by intraperitoneal injection at 24, 48, and 72 hours after inoculation, each caused a significant reduction in parasitaemia within 6 hours, becoming most marked at 12 hours. The trypanocidal potency of actinomycin D was revealed when the maximally tolerated dose was injected subcutaneously at one time. Despite its ability *in vitro* to interfere with several metabolic pathways of *T. equiperdum*, pactamycin was almost inactive *in vivo* (Jaffe, 1967).

According to Barrowman (1976) MSbE was able to sterilize *T. equiperdum* infections in experimental horses (by blood inoculation). The use of the drug in naturally infected horses, where the parasite had exerted its tissue and CSF penetrative abilities, was shown to be ineffective at the dosage used.

The study conducted to identify a trypanocidal factor against *Trypanosoma equiperdum* in normal human serum has shown that it has a trypanolytic activity. Human serum given to *T. equiperdum* infected mice caused a rapid decrease in the number of circulating trypanosomes and protection from lethal infection, while such activities were not found in sera from a range of animals susceptible to infection (Verducci *et al.*, 1988). Drugs induce the complete loss of kinetoplast DNA. A fully kinetoplastic strain of *T. equiperdum* has been made dyskinetoplastic by successive treatments of infected rats with Berenil. The complete loss of kDNA is stable for at least 6 additional passages in rats, in the absence of Berenil (Riou and Benard, 1980). Similarly a fully kinetoplastic strain of an antigenic variant of *T. equiperdum* has been made dyskinetoplastic by successive treatments of infected rats with ethidium bromide or acraflavine. After seven passages in the absence of the dye, the loss of kinetoplast DNA (kDNA) is stable and complete (Riou, Belant and Benard, 1980).

Recently a new trypanocide Cymelarsan has been shown to be effective for the treatment of *T. evansi* in camels at 0.3 or 0.6mg/kg body weight. It is also believed to be active against the trypanozoon sub-genus including *T. equiperdum* (Zelleke, Kassa and Abebe, 1989).

### **Drug resistance**

Drug resistance is usually considered to arise when inadequate dosages with the same, or similar, drug are administered repeatedly. The persistence of trypanosomes in the blood, or their detection in the body, subsequent to treatment at the recommended dosage indicates drug resistance (Stephen, 1986). The appearance

of trypanosome strains resistant to the limited number of trypanocides available is an increasing problem for the chemotherapy and chemoprophylaxis of livestock. Moreover the lack of new trypanocides appearing on the market for more than two decades has exacerbated the situation in the control of trypanosomiasis (Williamson, 1970; Williamson, 1976; Losos, 1986). Attempts at rational drug design are still in their infancy and it may take many years before new drugs are developed. However, better use of existing drugs by rational combination chemotherapy could help to fill this gap (Jennings, 1990). Studies have indicated that abnormal antibody responses may be the cause of drug resistance in immunologically compromised hosts (Schnitzer *et al.*, 1961; Osman, Jennings and Holmes, 1992 ).

*Trypanosoma evansi* and *Trypanosoma equiperdum* clones originating from different countries tested for drug sensitivity study, *in vivo* and *in vitro* against diminazine aceturate, suramin, Mel Cy, quinapyramine and isometamidium showed that clones selected for resistance to diminazine aceturate were not cross resistant to suramin and isometamidium. In contrast, the clones resistant to daminazine were shown to be more sensitive to quinapyramine than the normal strains (Zhang, Giroud and Baltz, 1992)

## Diagnosis

The demonstration of trypanosomes in the blood and tissue fluids is axiomatic, particularly if the effectiveness of therapy is to be assessed (Stephen, 1986) and in the case of tsetse-transmitted trypanosomes the combination of clinical signs, epidemiology and blood examination is often sufficient to establish a diagnosis, particularly in areas of high prevalence (Luckins, 1992). In *T. equiperdum* infections it can be difficult to demonstrate the parasite directly as its presence in the circulation is transient (Wassal *et al.*, 1991; Stephen, 1986; Hoare, 1972). However Barrowman (1977) reported his success in demonstrating the parasite in blood taken from horses in the early stages of the disease and considered the sedimentation technique to be

most useful. However, the examination of blood taken from chronic cases of unknown duration gave negative results.

Earlier studies in India showed that enormous numbers of *T. equiperdum* are to be observed in the vaginal mucus of a minority of mares affected with dourine at certain intervals during the course of the disease. Lingard (1906) stated that *T. equiperdum* only attains perfection in its development in the contents of cutaneous oedematous plaques of the horse.

As mentioned earlier the detection of this parasite in the blood of the natural host is frequently very difficult and because of this Watson (1920) stated that it is futile to search for *T. equiperdum* in the blood.

The first definitive diagnosis of *T. equiperdum* in South Africa was made in 1935 where smears were made of various organs from a sick mare which was killed and autopsied. A number of trypanosomes were identified in the mammary gland smears of this animal. This finding led to the close examination of recently infected horses and trypanosomes were found in centrifuged vaginal washings from a mare at autopsy, although smears from the organ were negative. Later trypanosomes were demonstrated in the vaginal washings from a living mare by the same technique, but all attempts to find the parasite in the blood failed. Notwithstanding the negative findings of blood smear examination it was decided to centrifuge the citrated blood and to examine the lower plasma level and the buffy coat. A number of trypanosomes were demonstrated by this method. However careful searching was necessary (Parkin, 1948).

Because of the uncertainty of finding this parasite in the tissues and its fleeting presence in the bloodstream of equines suffering from the disease, in practice this disease has been diagnosed indirectly on the basis of clinical signs and the Complement Fixation Test (CFT) (Hoare, 1972). The limitations of parasitological diagnosis have been the driving force for research into alternative techniques that provide indirect evidence of infection, namely immunodiagnostic techniques. The



CFT has played a major role in the eradication of the disease from many developed countries (Luckins, 1977; 1992).

Complement fixation tests find their greatest usefulness in regions where only one species of trypanosome occurs. Such a situation occurred in Canada with dourine in horses. The eradication programme in this country relied exclusively on this test to detect reactors. The test was brought into general use in 1913 and by 1920 the disease had been eradicated (Watson, 1915, 1920). Within the republic of South Africa the CFT was used and is still widely used for the diagnosis of dourine (Williamson and Herr, 1986).

CFT remains a useful test for the diagnosis of dourine except in areas where other trypanosomes of the subgenus trypanozoon (*T. evansi* and *T. brucei*) occur since such organisms have antigens in common with *T. equiperdum* (Barrowman and vanVuuren, 1976; Woo 1977; Williamson *et al.*, 1988; Brown, Hunter and Luckins, 1990).

However compared with modern immunoassays CFT is laborious and inconvenient (Anon, 1988) and is becoming less popular for the diagnosis of dourine and other diseases (Wassal *et al.*, 1991). Furthermore the test gives a large number of inconclusive results in the diagnosis of dourine, particularly with mule and donkey serum (Williamson and Herr, 1986). Watson (1920) reported some success with agglutination and precipitation test but, they were less satisfactory than the CFT tests developed by him.

An improvement in immunological diagnosis came with the introduction of primary binding assays for the detection of trypanosomal antibodies. The test measures directly the interaction between antigen and antibody rather than relying on a secondary reaction consequent upon the initial binding. The indirect florescent antibody test (IFAT) has been used extensively in the detection of trypanosomal antibodies in animals and man. IFAT has been used as a confirmatory test of dourine but the interpretation is very subjective and requires experienced workers and

sophisticated equipment for consistent results (Wassall *et al.*, 1991; Luckins, 1992). Research findings indicate that the simple antigen detection slide agglutination test which is designed for diagnosis of non-tsetse transmitted animal trypanosomiasis may be effective for *T. equiperdum* in the future (Nantulya, 1992).

*T. equiperdum* is a difficult parasite to grow in laboratory animals following subinoculation of samples of blood or tissue from horses and care should be taken in differentiating *T. equiperdum* from *T. evansi* and *T. brucei*. The background of the area or farm should be assessed carefully in order to have the clear epidemiological picture. Clinical manifestations are very specific and in most cases are prominent for both sexes although dourine is probably never seen as an acute infection in the true sense (Stephen, 1986).

In tropical Africa closely related species of *T. evansi* and *T. equiperdum* occur and so far, cannot be reliably differentiated. Little attention is paid to diseases of donkeys, and although horses with symptoms of paresis or oedema of the genitalia are common, a proper diagnosis is often not made (Uilenberg, 1992).

Compared to other techniques, enzyme-linked immunosorbent assays ELISAs are clearly the leading candidates for a number of reasons.

- 1) It is possible to produce defined antigens and monoclonal antibodies.
- 2) It is easy to automate and screen large numbers of samples.
- 3) With appropriate modification, it should be possible to adapt the assay for use under field conditions (Luckins, 1992).

In the diagnosis of *T. equiperdum* ELISA could prove to be very useful. It would be relatively easy to standardise between laboratories and an ELISA protocol could be adopted as the internationally approved test for equine certification (Wassall *et al.*, 1991).

The use of purified antigen in the direct ELISA for antibody detection has markedly reduced the quantities of antigen needed and this has emphasised the benefits of ELISA over IFAT and CFT for dourine diagnosis (Luckins, 1978, 1992;

Caporale *et al.*, 1981; Wassall *et al.*, 1991).

The next chapter describes a study conducted in Ethiopia using ELISAs to examine the prevalence of *T.equiperdum* in horses in a selected highland region.

## Chapter 4

### Investigation of *Trypanosoma equiperdum* in Ethiopia

The problem of dourine in Ethiopia has been recognised by local farmers for many years. However, the first official report of the disease was made after the Arsi Rural Development Unit requested the Tsetse and Trypanosomiasis Survey and Control Department to investigate a persistent disease problem in horses in the administrative regions of Arsi and Bale administrative regions. In response a team was sent to the area where the physical examination of 25 animals (17 mares and 8 stallions) was carried out and a detailed study conducted on five horses which were at that time thought to be in the acute stage of the disease. As a result of the study the disease was found to be dourine caused by *T. equiperdum* (Dagnachew, Shafo and Kelil, 1980). Following this result the team gave recommendations for further studies and for the control of the disease but no steps have been taken since then. However, control is likely to be difficult because equines are normally allowed to run unrestricted on the open range.

The Arsi and Bale people like any others in the remote areas of Ethiopia, where communications are poor, use horses as pack animals, for draft work on farms (plate 4.1) and for riding as well. For these reasons equines in Arsi and Bale are extremely numerous and this makes dourine potentially a very important disease in this part of the country.

The present study was conducted to investigate the incidence of *Trypanosoma equiperdum* in the horse population of Arsi and Bale regions in order to assess the importance of this disease. The study consisted of a field investigation to assess the background of the disease using questionnaires; examination of clinical cases of dourine among the equine population in the area; and collection of blood samples from clinically affected and normal horses. Later the blood samples were examined using indirect immunosorbent assays (ELISAs) for evidence of *T. equiperdum* antibodies and antigen in the test sera. Based on the results of the double sandwich

**Plate 4.1** Horses Engaged in Threshing Wheat in the Field



ELISA, fresh blood and genital discharges were inoculated into laboratory animals (horses, rabbits) from antigen positive horses.

All the necessary chemicals, and reagents and materials for the field investigation, sample collection and laboratory work were sent by air freight to Ethiopia whilst the biologicals were taken by hand by the investigator in thermos-flasks filled with dried ice.

On arrival in Ethiopia communication was made with the national and local authorities to obtain permission for the investigation work and to select the study areas. The selection was based on the criteria that the sites should be outside the known tsetse-infested areas, have no camels or known *T. evansi* cases and possibly have previous records of *Trypanosoma equiperdum*. In addition efforts were made to identify laboratories with ELISA facilities in the Veterinary Services Department and permission was obtained to conduct the test in either the Debre Zeit or Shola laboratories. For convenience the Shola laboratory was chosen. After collecting 25 horse sera from the American Baptist Mission Veterinary Clinic in Addis Ababa, where there was no known case or report of dourine, the ELISA was established and the sera used as negative reference sera.

## **Field investigation and blood sample collection**

### **Materials and Methods**

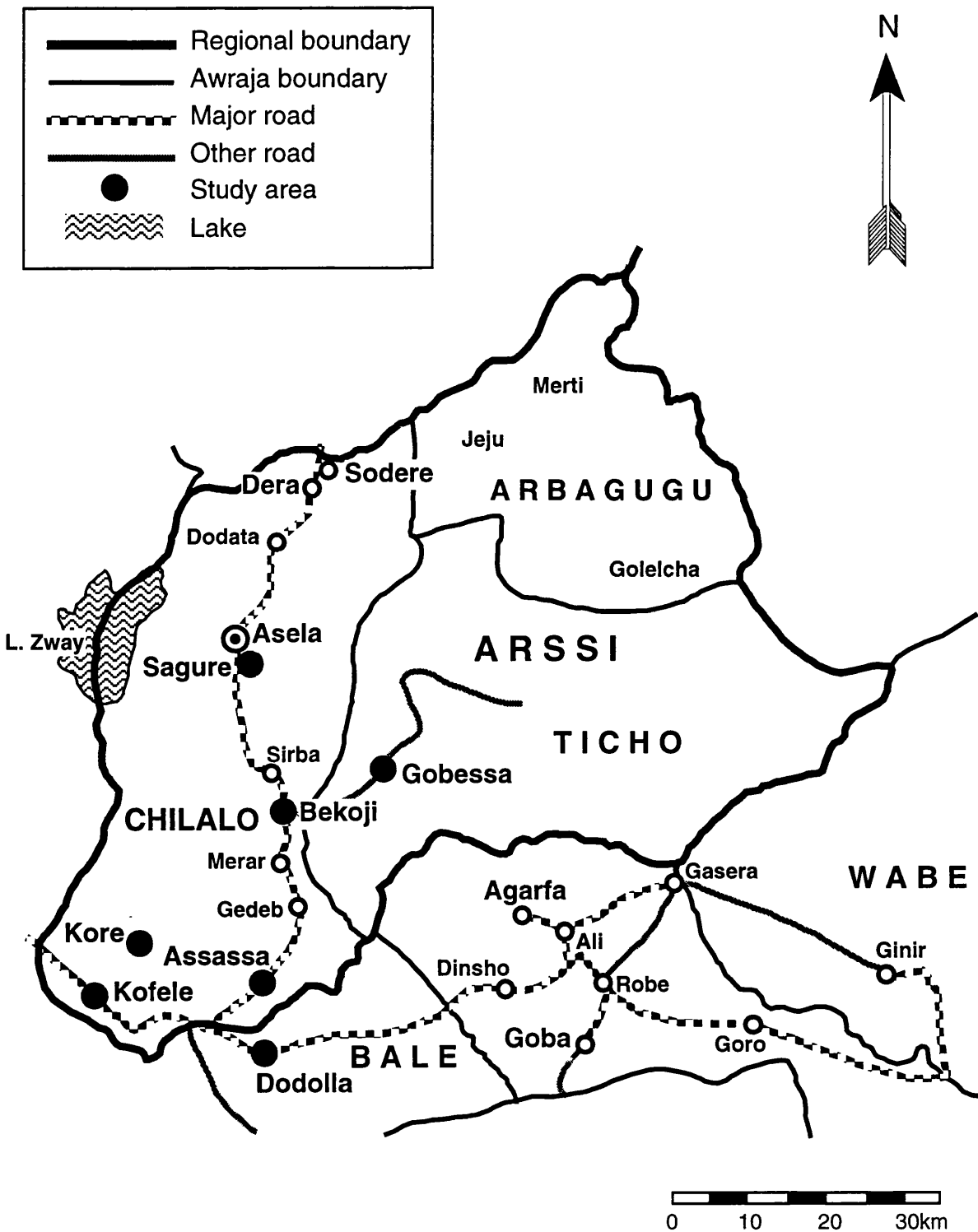
#### **Study Areas :**

The selected study areas were in Arsi and Bale administrative regions which are located in the south-east part of the country (Plate 4.2 and Plate 4.3 ). The equine populations were estimated to be 802,000 and 101,000 respectively in the two regions and dourine was regarded as a serious problem in both regions. There was a strong request by the Veterinary Services Department to include more areas in this study. However, constraints of time and technical materials limited the study to the following seven sites (Table 4.1).

**Plate 4.2** *Trypanosoma equiperdum* Distribution.



**Plate 4.3** Study Areas in Arsi and Bale Regions.





**Table 4.1.** Selected study sites for the investigation of *T. equiperdum*.

Study area	Region	Altitude	Distance from Assela in k/m
Sagure	Arsi	2460 masl	23
Bekoji	"	2810 "	58
Shirka (Gobessa)	"	2330 "	94
Assassa	"	2370 "	108
Dodolla	Bale	2440 "	125
Kofele	Arsi	2680 "	195
Kore	Arsi	2730 "	220

(masl = metres above sea level)

**Horses** : The local horses of both sexes that were selected were living under traditional management of free grazing. In most cases sexually mature horses were selected randomly for physical examination and blood sample collection.

**Questionnaire** : A questionnaire was designed and copied for use in the field (Figure 4.1).

**Protocol:**

The following steps were taken in the investigation.

1. Dissemination of information about the aims of the study to local farmers and requests to bring their horses to the selected sites for clinical examination and blood sample collection.
2. The collection of detailed information of each study area and the history of dourine in equines by interviewing the farmers who lived locally and the local Veterinary personnel.
3. The random selection of horses regardless of their clinical symptoms and body condition. However, the age group was considered in order to select only sexually matured horse but more females appeared in the selected groups by chance.
4. The collection of a blood sample from the jugular vein of each horse.
5. Five hours after collection and following coagulation the blood samples were centrifuged and the sera separated and stored for serological diagnosis.

**Collection of Local Information**

The field study began on November 18, 1993. There was one laboratory technician and a driver in the team. The main station was in Assela which has a well furnished regional laboratory established by the Swedish International Development Agency (SIDA). Based on the information given prior to our arrival the Veterinary personnel as well as the farmers were eagerly waiting for the team and field visits started on the next day.

**Figure 4.1 Questionnaire for Investigation of *Trypanosoma equiperdum* in Ethiopia**

Country.....Region.....Province.....

District.....Locality.....

Ecological Zone.....

OwnersName.....Address.....

Species of the animal.....Name if any.....

Age.....Sex.....Distinguishingmarks.....

**Veterinary Records**

Reproductive status of the animal.....

If female how many months or years since gave birth or aborted.....

.....What disease has the animal had in the last years including  
the present.....

.....

.....

.....

.....

What treatment has the animal received during its past.....

.....

Present clinical symptoms if any.....

.....

.....

.....

Samples taken.....Purose of sampling.....

Date of sample collection.....Initial.....

According to the local people and the Veterinary personnel in Arsi region dourine is known locally as "Lappessa Hidakuta" or "Lappessa Dudakuta" which means backbone breaker in the local Oromo language and in Bale region it is known as Dirressa. The farmers from both regions stated that the first indication in affected horses is that the animal becomes very slightly slack in the hind quarters and straddles the legs. Females shows moistness around the vulva and buttocks. Eventually the conditions become so serious that the animal lies down and has difficulty in rising. In most cases they die after suffering for some time. Some farmers mentioned that if after becoming sick, they were given a change of feed or put on green feed and barley they picked up for a time, but the improvement was not lasting. The farmers claim that the disease is the major equine problem in these two regions and causes high morality and many abortions in females. It has a seasonal character which coincides with the breeding season (February to May).

In general it was not possible to trace the origin of the spread of the disease or to associate the first occurrence of the disease with any particular event in the past. In spite of that, the peasants in Bale Administrative region are of the opinion that the disease has spread to their area from Arsi in the past two decades. This might be true because hundreds of horses cross from Shoa via Arsi to Bale region for marketing (Plate 4.4). The Veterinary personnel in the study areas are very concerned that the disease is becoming endemic in new areas.

## **1. Clinical Examination**

Large numbers of equines were seen on market day in Assela, the capital town of Arsi. Hundreds of horses, mules and donkeys were there from different corners of this region. In the population quite significant numbers horses of both sexes showed various signs that are described in most of the literature as typical of dourine (from the acute to chronic form) but no blood samples were collected. However, similar clinical signs were observed in horses in each study area.

**Plate 4.4** Horses Crossing from Shoa to Arsi and Bale Regions for Marketing.



Mare : Discharge from the vagina and swelling of the external genitalia was apparent and this was prominent on the vulva and clitoris (Plate 4.5). The vaginal mucous membranes were congested and in some cases the floor of the vagina was covered with a mucopurulent discharge which sometimes escaped through the vulva to the outside (Plate 4.6). The hips and tails were wet. De-pigmentation of the vulval lips also occurred. In some mares frequent urination was observed and there were ulcers on the labia and clitoris.

Stallion : In a few cases oedema of the scrotum and prepuce was observed and patchy de-pigmentation of the prepuce was clearly seen. Increased sexual desire and frequent erection and micturation was also observed.

Both sexes : A number of horses had a straddled gait, and the hind legs were far apart particularly when trying to walk forward (Plate 4.7). Lameness in one or both hind legs occurred and there was restlessness of the hind legs. Some cases were paraplegic. In paraplegic horses muscular atrophy in the gluteal region occurred to varying degrees. In a few cases there was difficulty in rising and lying down and this was normally followed by paralysis and death. In addition to these clinical signs suspected animals were frequently emaciated and in poor condition. In a few cases conjunctivitis was observed.

Although it was difficult to reach any conclusion from the clinical signs it was found useful to categorise the clinical signs in a way that could be used later in comparison with other diagnostic results. For this reason all the 309 horses clinically examined in the two regions were categorised into one of four groups. These were,

group 1, horses without any specific clinical signs.

group 2, horses with modest clinical signs but not specific for dourine.

group 3, horses with clinical sign of dourine but limited to the genital organs and skin.

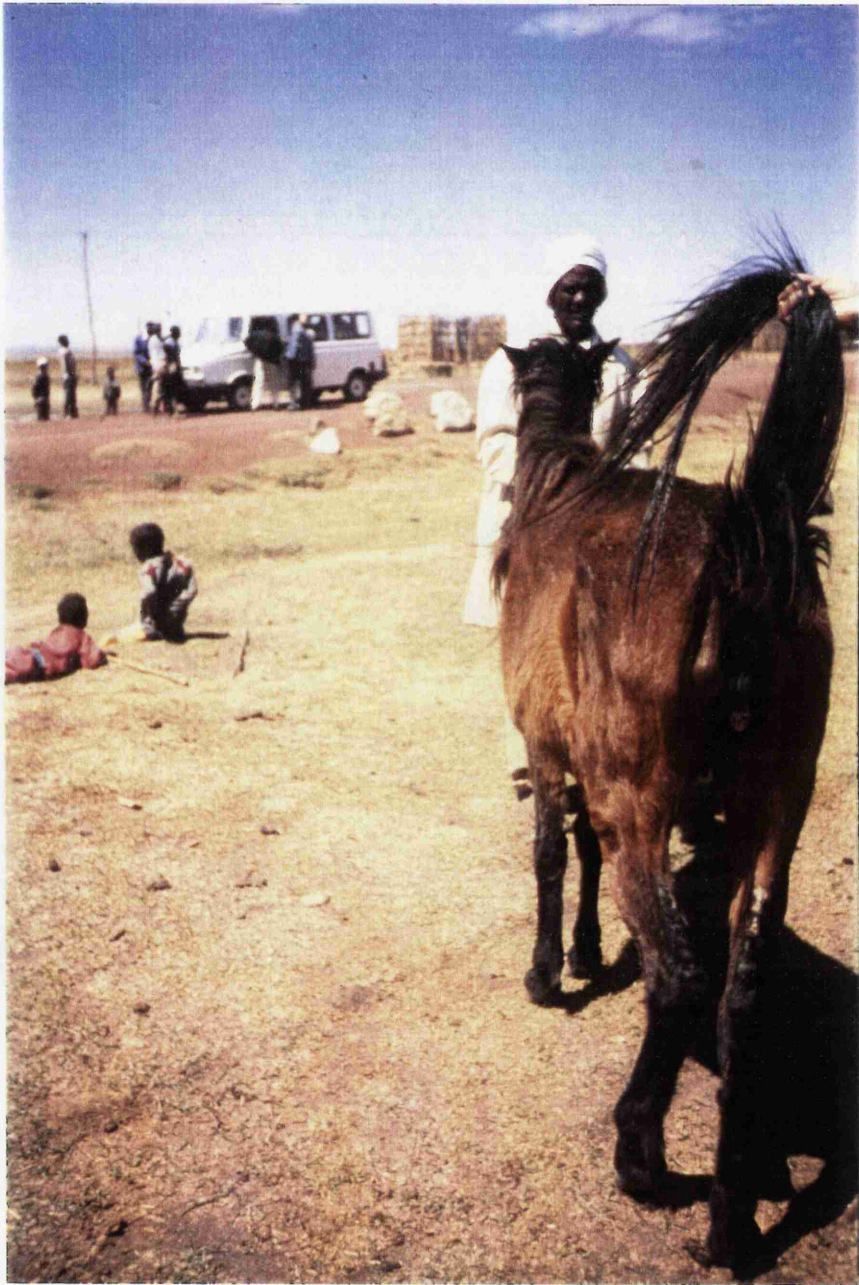
group 4, horses with typical clinical sign including the limbs and nervous disturbances.

**Plate 4.5** Swollen Vulva with Slight De-pigmentation.





**Plate 4.6** Mucopurulent Vaginal Discharge on the Lips of the Vulva





**Plate 4.7** A horse Showing Difficulty in Walking with Characteristic  
Gait in Hind Limbs.



## **Blood sample collection**

After the interview of the owners about their horses' previous health record and the clinical examination, blood samples were collected from the jugular vein of each horse (Plate 4.8). The collected blood was centrifuged using a clinical centrifuge, (ICE, USA) and the serum removed using a plastic pipette (Alpha Labs). The separated serum from each animal was placed in two numbered 1.5ml micro centrifuge tubes and closed tightly before placing in the deep-freeze (Electrolux model TC 1150) at -20°C. Sera collected from different sites was kept separately in self-seal polythene bags labelled with the collection site and date of collection).

During the field investigation sera from 309 horses (85 males and 224 females) was collected at seven sites out of which 85 were from male and 224 from female horses (see Table 4.2.). These sera were brought to Addis Ababa in a frozen state and kept at -20°C until tested using the indirect enzyme linked immunosorbant assays (ELISAs).

**Plate 4.8** Blood Sampling from a Horse.



**Table 4.2** Number of blood samples collected from the study areas.

Study Areas	Number of Blood samples
Sagure	47
Shirka	81
Bekoji	43
Dodolla	55
Assassa	28
Kofelle	25
Dodolla	30

## **Enzyme Linked Immunosorbant Assays (ELISAs) for the diagnosis of *T. equiperdum***

Definitive diagnosis of trypanosomiasis requires the correct recognition of clinical signs and the demonstration of the parasite. However in the case of *Trypanosoma equiperdum* infections the clinical signs are not specific and can be confused with those of other diseases. Symptomless infections can also occur. It is also very difficult to detect the parasite in the affected tissues or to find them in the blood. However, recent developments in ELISA technology for the detection of circulating antibody and antigen (Luckins, 1977, 1978, 1979, 1992; Nantulya, 1989, 1990; Voller *et al* 1977; Voller, 1979) offer new opportunities in the diagnosis *T. equiperdum* infections. The sera collected from Arsi and Bale regions of Ethiopia was tested using ELISAs developed for the detection of *T. evansi* infections by Dr. A.G. Luckins at the CTVM but which were known to cross-react with *T. equiperdum*. This was the first opportunity to apply these assays to natural infections of *T. equiperdum* in African horses.

### **II Indirect ELISA for the detection of *Trypanosoma equiperdum* antibody**

Initially the indirect ELISA test was conducted in Ethiopia using the facilities of the Central Disease Investigation Laboratory at Shola, Addis Ababa in order to screen the 309 test sera that had primarily been collected from local horses of both sex from the seven sites in Arsi and Bale administrative regions of Ethiopia. Later the sera was brought to Glasgow. It was a condition of the import licence that the sera was heated to 56°C in a water bath for 30 minutes prior to importation.

#### **Principle:**

The system adopted was the indirect ELISA (Fig. 4.2). *Trypanosoma equiperdum* antigen was prepared from infected rat blood and absorbed to the solid phase (Immulon 1, Dynatech laboratories) during coating. Then the test sera was added and the *T. equiperdum* antibody allowed to bind onto the coated plate.

Thereafter the secondary antibody which is conjugated to an appropriate enzyme (anti-horseradish peroxidase) was added to detect the bound *T. equiperdum* antibody. The substrate (TMB) and hydrogen peroxide was added and the reaction stopped with H<sub>2</sub>SO<sub>4</sub>. The plates were read photometrically and the optical density was used as an indicator value of the antibody level.

### Materials and Methods

**Antigen** : *Trypanosoma equiperdum* antigen prepared from infected rat blood was kindly supplied by Dr. Phipps of the Central Veterinary Laboratory ( CVL ), Weybridge.

**Positive control serum** : This was also kindly supplied by Dr. Phipps from horses infected with *T. equiperdum* .

**Negative Control serum** : This was obtained from a normal horse at the Veterinary School, Glasgow University which never had the exposure to any species of trypanosome.

**Conjugate** : Anti-horse IgG (whole molecule) peroxidase conjugate developed in rabbits was obtained from SIGMA chemical company Ltd.

**TMB** : 3,3',5,5'-Tetramethyl -benzidine was obtained from SIGMA company Ltd.

**Hydrogen peroxide** : 30% H<sub>2</sub>O<sub>2</sub> also obtained from SIGMA

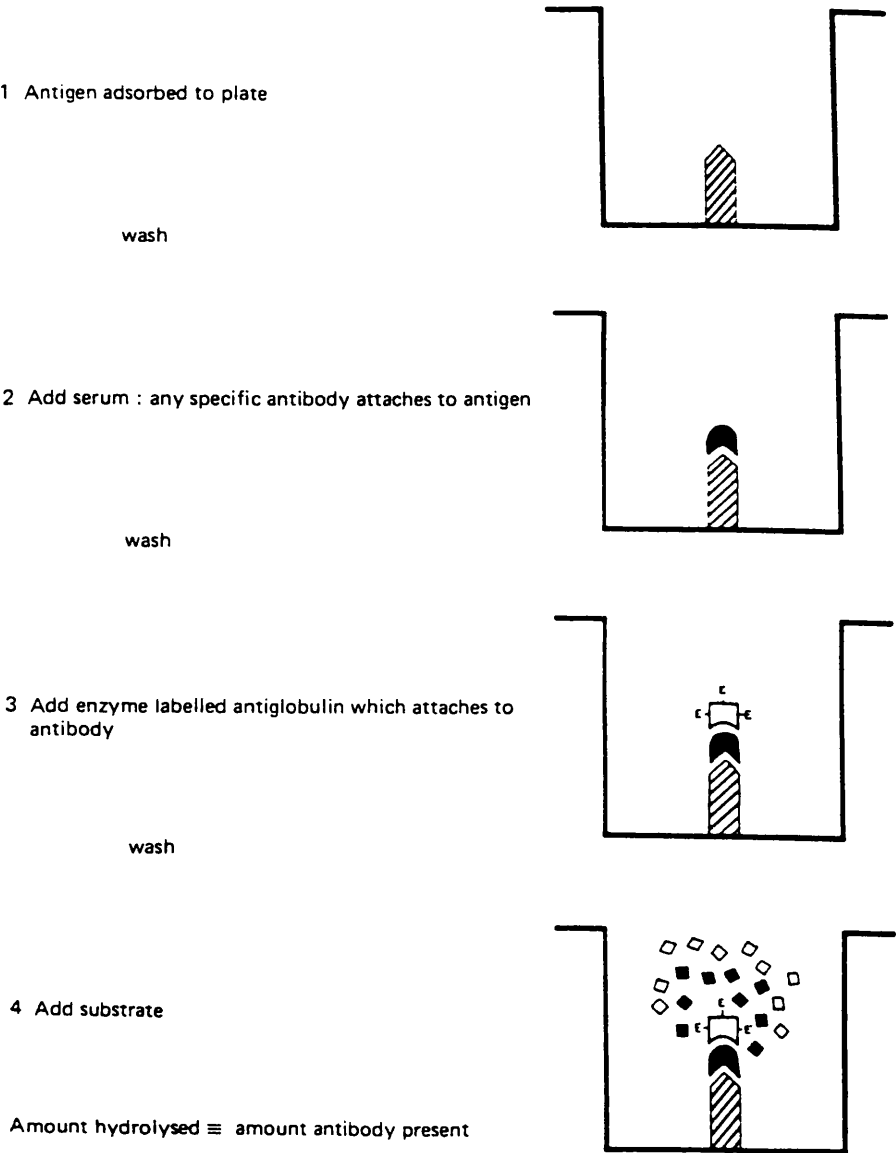
**Microtitre Plates** : Immulon 1 microtitre plates were obtained from Dynatech and plate sealers from ICN Flow.

### Methods Employed

The following buffers were prepared before the onset of sample testing.

- carbonate /bicarbonate coating buffer pH 9.6
- washing buffer (phosphate buffered saline + 0.05% Tween 20) pH 7.2
- diluting buffer (PBST + 1% bovine serum albumin) pH 7.2
- citrate/ acetate substrate buffer pH 6.0
- 2M sulphuric acid

**Figure 4.2** The indirect ELISA for assay of antibody



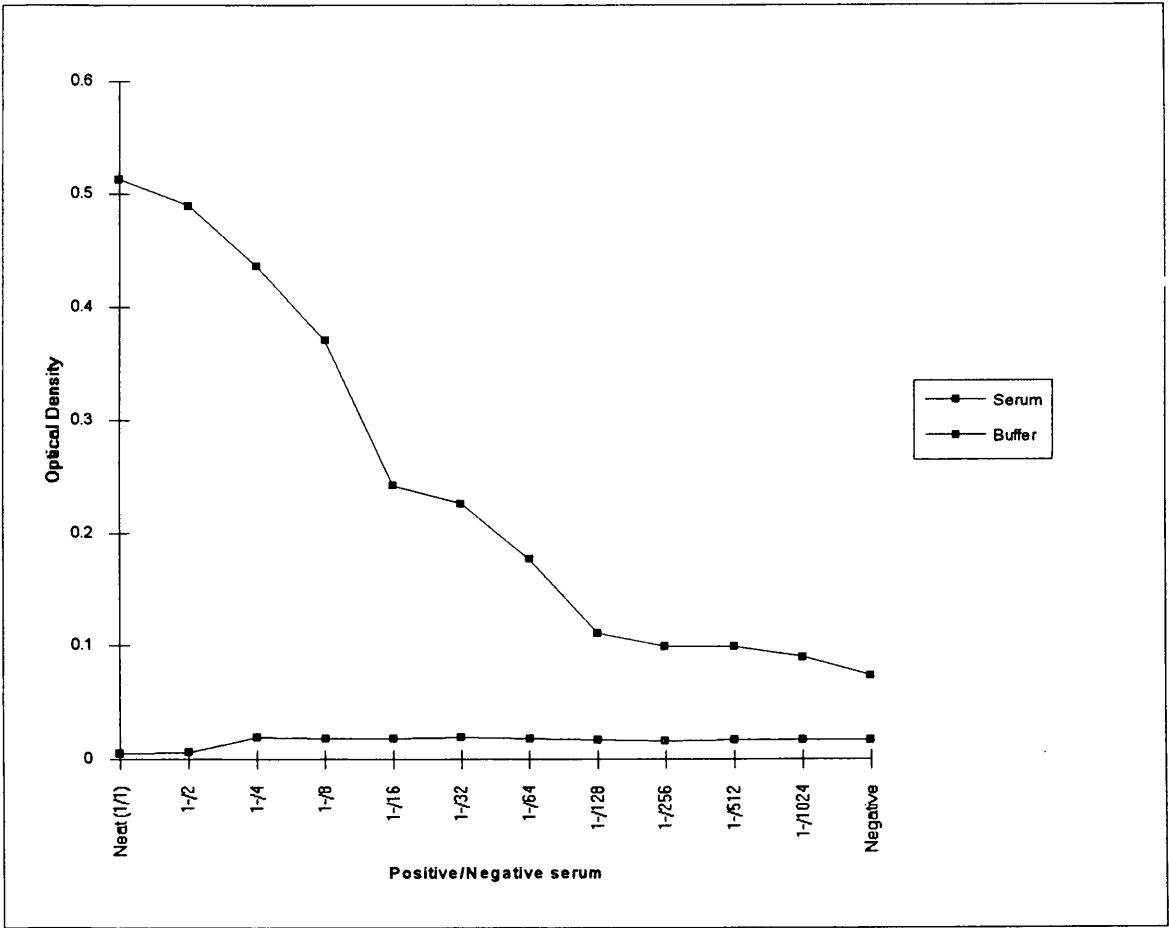
Voller *et al.* (1979)

**Figure 4.3** The plate lay out designed for testing the horse sera as distant duplicates.

	1	2	3	4	5	6	7	8	9	10	11	12
A	C++	1	9	17			C++	1	9	17		
B	C++	2	10	18			C++	2	10	18		
C	C+	3	11	19			C+	3	11	19		
D	C+	4	12	20			C+	4	12	20		
E	C-	5	13				C-	5	13			
F	C-	6	14				C-	6	14			
G	Cc	7	15				Cc	7	15			
H	Cc	8	16				Cc	8	16			



**Figure 4.4** Dilution of Positive /Negative serum to select C++ and C+ controls



**Reference sera:** As shown on the plate layout (Fig. 4.3), strong positive (C++), weak positive (C+), negative (C-) sera and no sera (Cc) were used in quadruple wells as reference for the test sera. The C++ and C+ controls were prepared by serial dilution of the positive control serum in the negative control serum. The dilutions selected were 1/4 and 1/40 respectively (Fig. 4.4).

**Titration :** To find out the optimal working dilution of the antigen sera and conjugate, titrations were conducted and at the optimal dilution selected as follows.

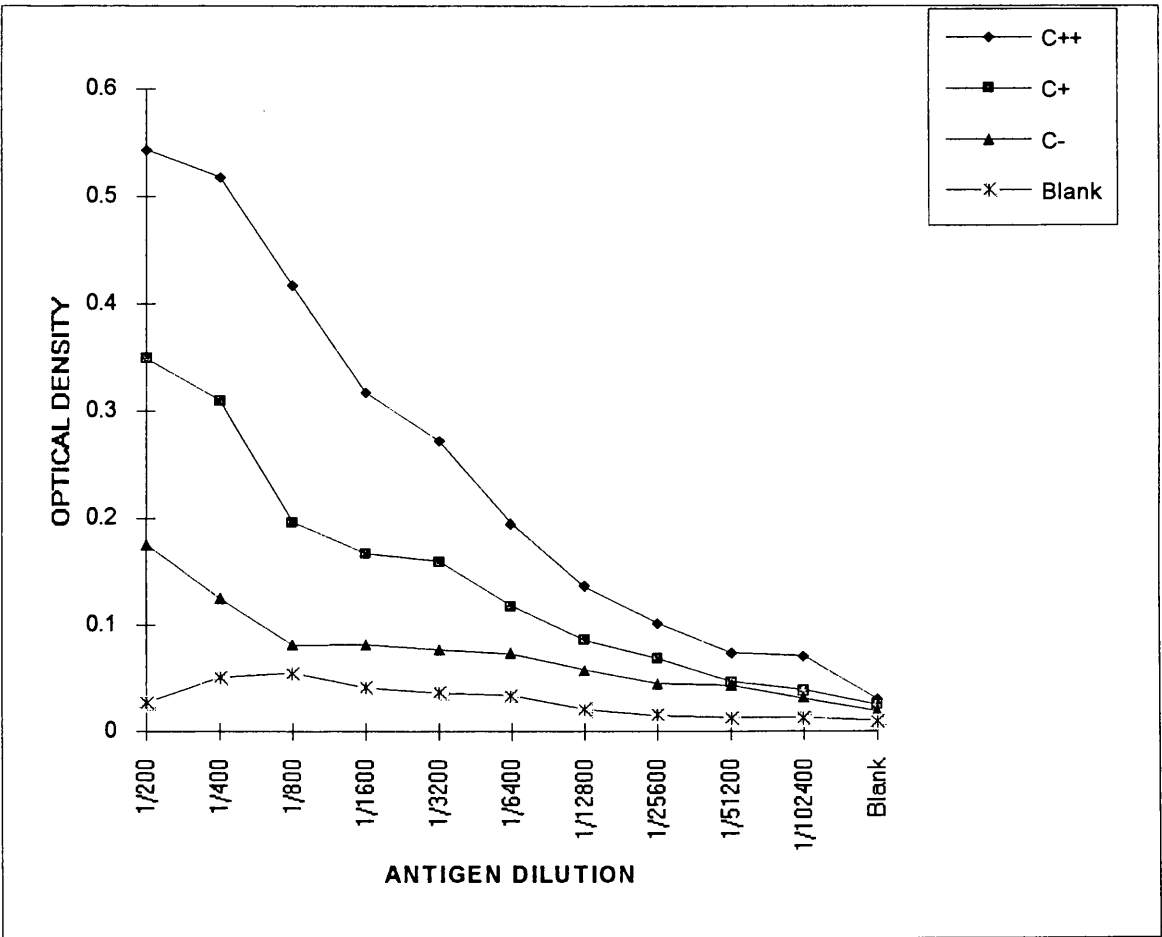
Microtitre plates were filled with coating buffer (100ul/well) then *T. equiperdum* antigen diluted 1/50 (20ul antigen + 980ul coating buffer) was added 100ul/well to column 1. Doubling dilutions were made across the plate to column 11. Column 12 contained 100ul coating buffer only. The remainder of the procedure was as described for the routine ELISA. This procedure was also used to determine the optimal working dilutions for the control serum and the anti-horse IgG.HRP conjugate.

**Antigen titration:** The optimal working dilution for the antigen was selected as 1/800 (Fig. 4.5).

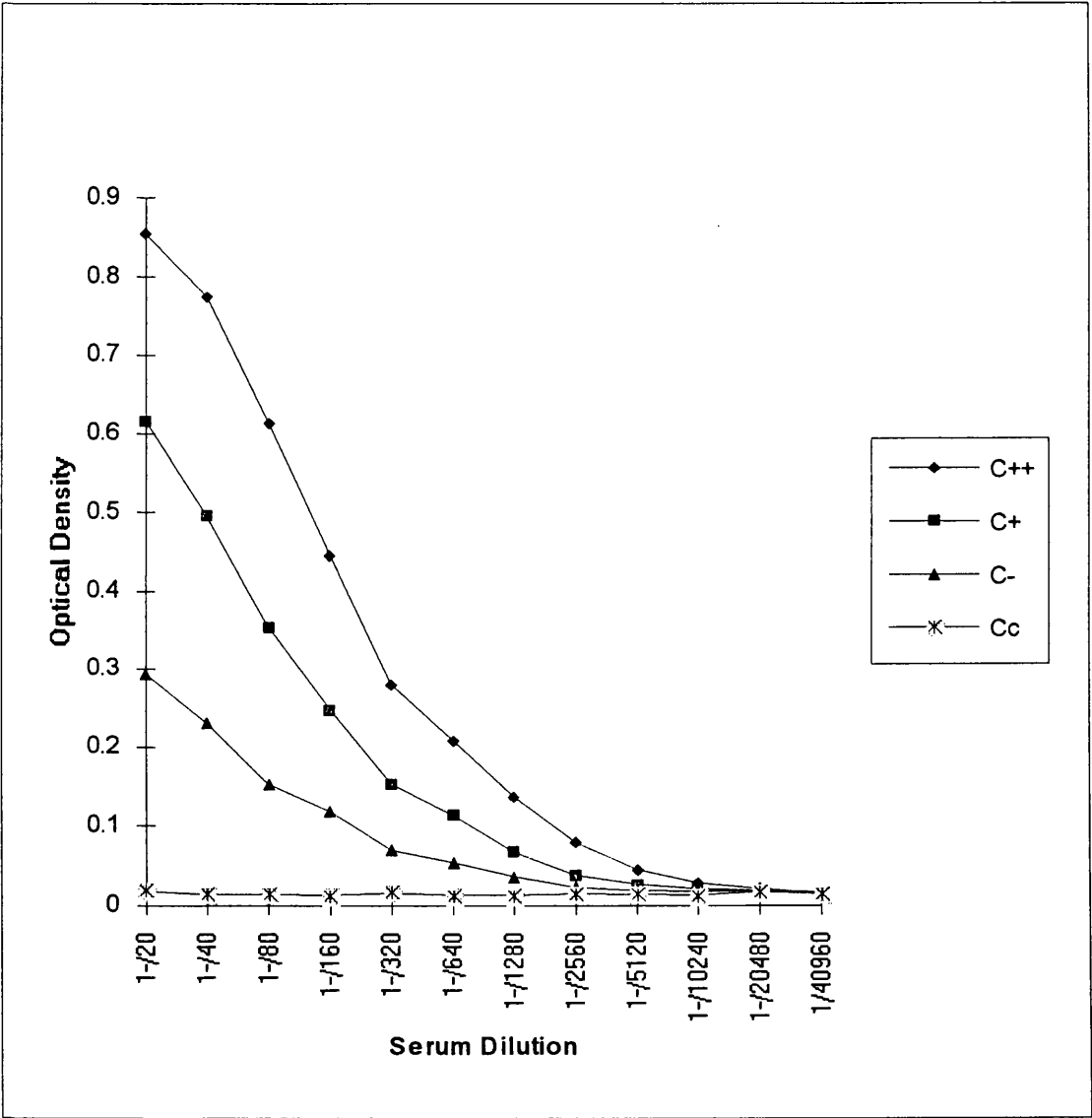
**Control Sera Titration:** The optimal working dilution was selected as 1/200 (Fig. 4.6).

**Anti-horse IgG.HRP titration:** The optimal working dilution of the conjugate selected as 1/15000 ( Fig. 4.7).

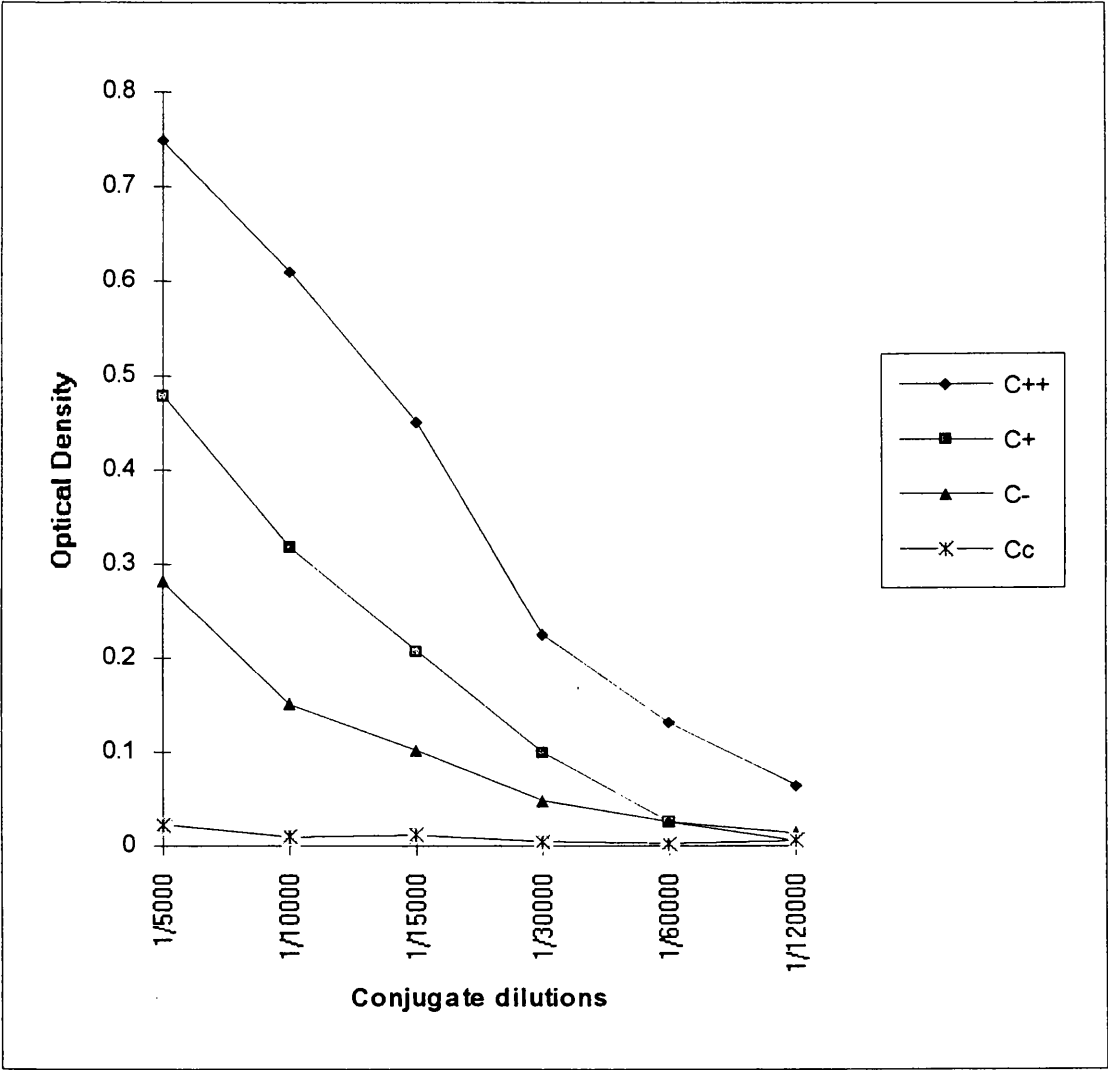
**Figure 4.5** Titration of *T. equiperdum* Antigen for Indirect ELISA



**Figure 4.6:** Titration of control sera for indirect ELISA.



**Figure 4.7** Titration of conjugate (Anti-horse IgG) for indirect ELISA.

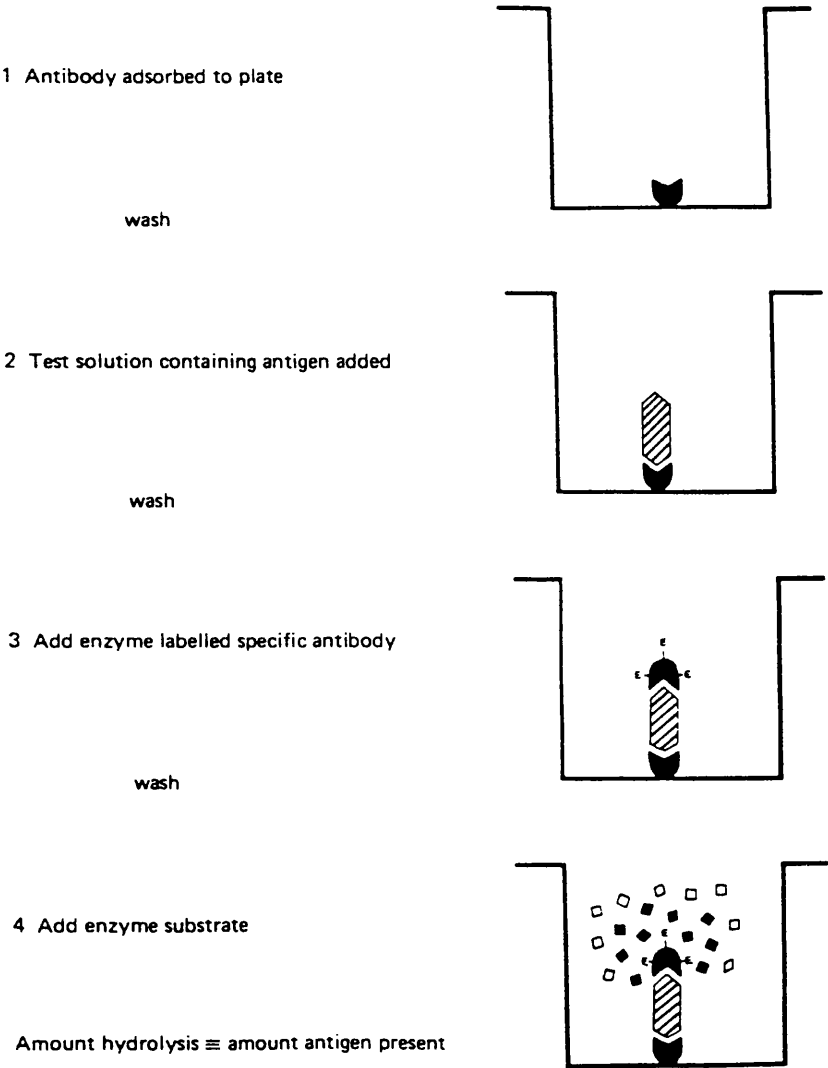


## Test procedures

*Trypanosoma equiperdum* stock antigen was diluted 1/800 in coating buffer and 100ul added to all 96 wells of each microtitre plate. The side of the micro plate was gently tapped to ensure that the antigen was evenly distributed over the bottom of each well and the plate was sealed with a plate sealer and left overnight at 4°C.

The next day the contents of the coated microtitre plates was discharged into a sink and washed five times and blotted dry on a sponge towel. Diluting buffer was added into the wells to avoid non-specific binding then the sealed micro plates were tapped and placed on a shaker - incubator at 37°C for 30 minutes. After 30 minutes following washing and tapping dry, control and test sera which had been vortex mixed were diluted 1/200 in diluting buffer and added to each well according to the plate layout. The sealed microtitre plates were shaken for 30 minutes at 37°C. The plate was washed and tapped dry as before, then conjugate at 1/15000 dilution was added to the plate (100ul/well). The microtitre plates were incubated for 30 minutes at 37°C. TMB was prepared for use 15 minutes before the end of the conjugate incubation. This was to allow the TMB to reach 37°C in a water bath before use. After washing and drying the micro plates, TMB solution was added to all 96 wells (100ul/well). A blank plate had 100ul of the substrate added to all 8 wells in a column. The plates were shaken in the incubator for 15 minutes and reaction stopped by adding 50ul of 2M sulphuric acid to all 96 wells of the micro plate and also the column on the blank plate. The micro plates were read photometrically on Tetertek multiscan plus microplate reader using 450nm filter.

**Fig 4.8** The configuration of double sandwich ELISA for measuring antigen.



Voller et al (1979).

## **Sandwich ELISA for the detection of *T. equiperdum* antigen**

### **Principle:**

This method was used to detect *T. equiperdum* antigen in horse sera. Monoclonal antibody against *T. evansi* prepared in mice and which was specific to *T. brucei* subgroup trypanosomes was absorbed to the solid phase during a coating step and later test sera containing trypanosome antigen was added to the coated plates and allowed to bind. Rabbit anti-*T. evansi* IgG, conjugated with horseradish peroxidase, (HRP) was added in order to detect the bound trypanosome antigen. After which substrate solution was added and followed by sulphuric acid. The OD value was measured for each sample and used to determine the level of trypanosome antigen present in the sera.

### **Materials and Methods**

**Monoclonal Antibody (MoAb)** Monoclonal antibody specific for *Trypanosoma brucei* was kindly supplied by Dr. Luckins of the CTVM, Edinburgh.

The other reagents were the same as those used in the antibody ELISA with the exception of the conjugate which was the same MoAb conjugated with HRP, also supplied by Dr. Luckins.

**Microtitre Plates :** Immulon 2 microtitre plates (Dynatech).

**Reference Sera:** As in the antibody assay the positive control serum was diluted in the negative serum as previously described. Dilutions of 1/4 and 1/32 were selected for the C++ and C+ respectively (Fig. 4.9).

### **Titration:**

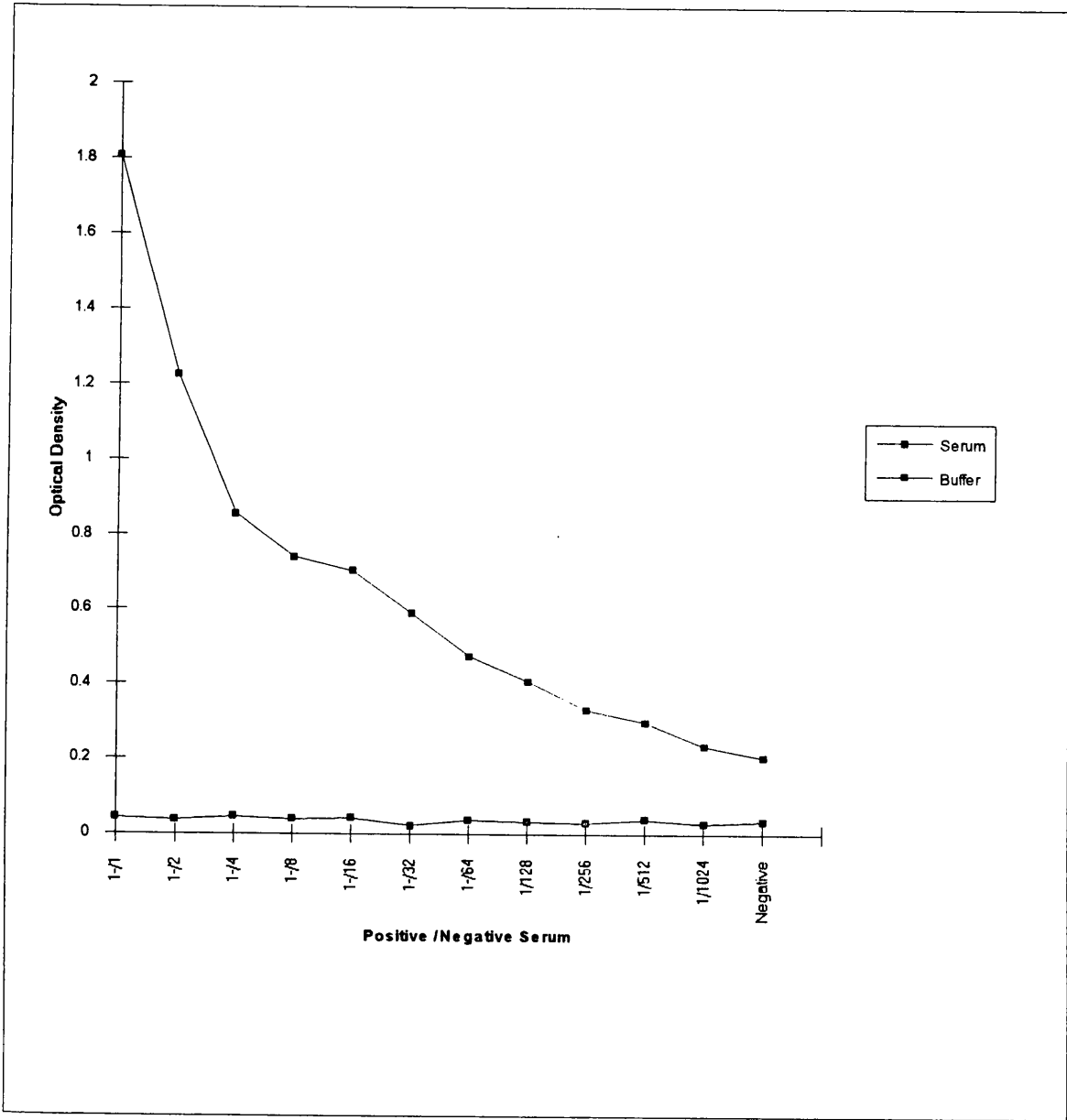
**Control Sera:** The titrations showed 1/20 as the optimal working dilution (Fig. 4.10).

The monoclonal antibody and the conjugate were used at the dilutions recommended by Dr. Luckins i.e. 1/400 for the monoclonal antibody and 1/4000 for the conjugate.

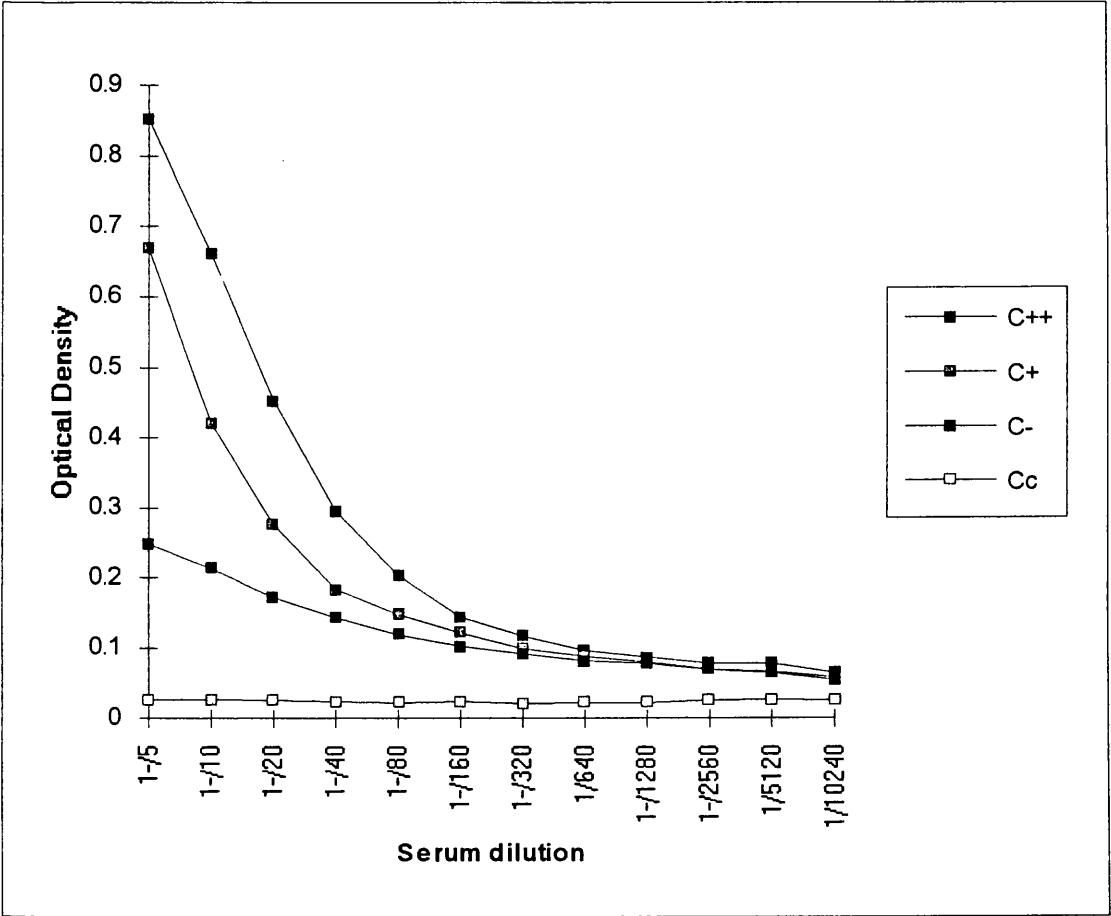
The plate design was the same as that for the antibody ELISA.



**Figure 4.9** Dilution of Positive/Negative Serum to select the C++ and C+ controls



**Figure.4.10** Titration of control sera for sandwich ELISA



### Test procedure

The aliquot of *T. evansi* MoAb stock was vortex mixed and diluted 1/400 in coating buffer. 100ul was added to all into 96 wells of Immulon 2 microtitre plates. The plates were tapped to ensure MoAb evenly distributed over the bottom of each well and then sealed with microplate sealer and left overnight at 4°C. The next day the contents were discharged into a sink and the micro plate washed five times as before then tapped-dry on sponge towel. 100ul diluting buffer was added to 96 wells and shaken on the shaker - incubator at 37°C for 30 minutes. Following washing and drying the plate as before control and test sera were vortex mixed then diluted 1/20 in diluting buffer and 100ul was added per well following the plate lay out (Fig.4.3). The plate was sealed and shaken on the incubator for 30 minutes. Conjugate (anti - *T. evansi* IgG HRP) was vortex mixed then diluted 1/ 4000 in diluting buffer immediately before use. After washing and drying the plate, 100ul was added to each well. The remaining steps of the ELISA were the same as those used in the antibody ELISA.

Based on OD readings all the samples with coefficient variations above 12% between duplicates were rejected and retested again.

**Data evaluation**

The results of both tests were analysed using the procedures described by Wright *et al.* (1993) and the manual prepared by FAO/ IAEA (1992) as follows.

a) percent positivity (PP) values which were used for quality assurance (QA) were calculated as follows:

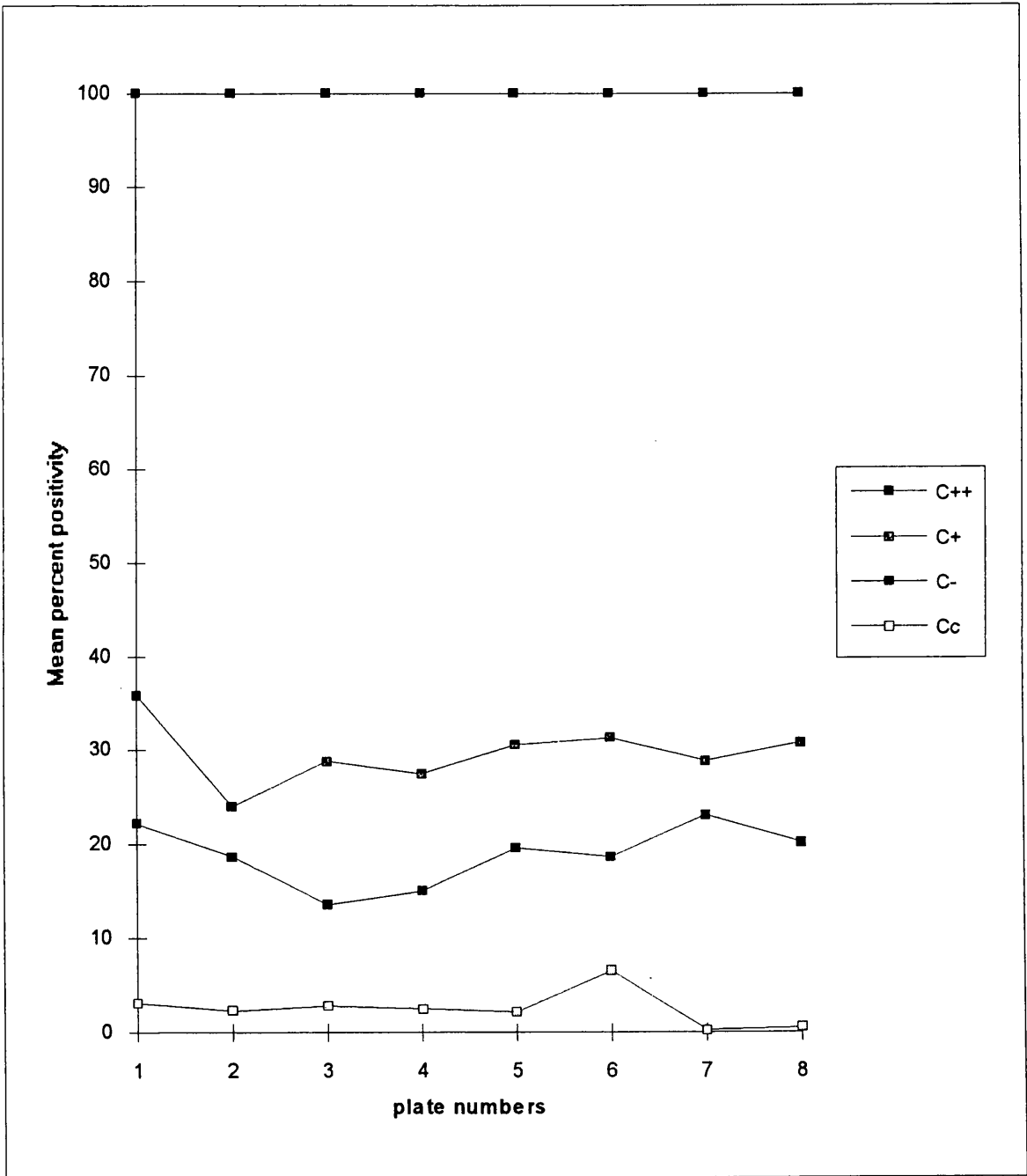
$$\frac{\text{Replicate OD value of each control}}{\text{Mean OD Value of C++ Control}} \times 100$$

b) percent positivity (PP) values used for acceptance of test sera data for diagnostic interpretation were calculated as follows:

$$\frac{\text{Replicate OD Values of the Test Serum}}{\text{Mean OD Value of C++ Control}} \times 100$$

Quality assurance for the control sera are shown in Figures 4.11 and 4.12 for the antibody ELISA and Figures 4.13 and 4.14 for the antigen detection ELISA.

**Figure 4.11** Quality assurance of the control sera for indirect ELISA.



**Figure 4.12** Mean control OD values of each plate used for antibody detection.

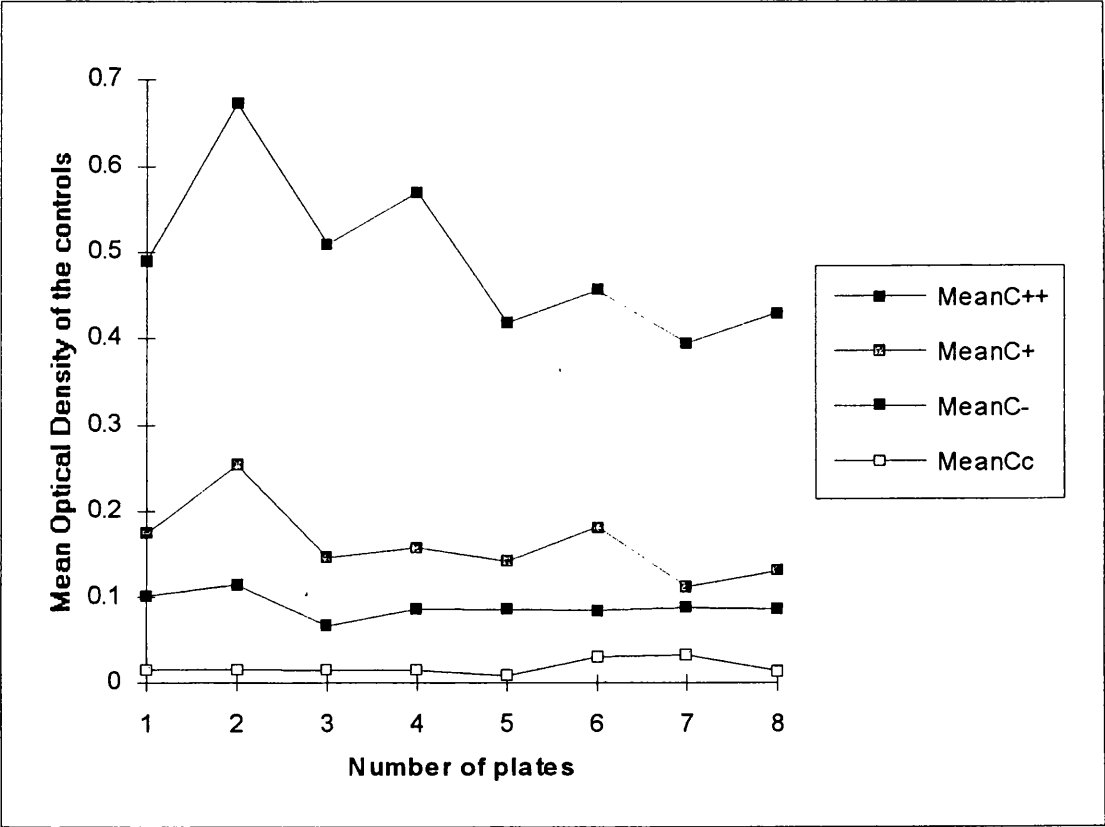


Figure 4.13 Quality assurance for control sera in the detection of *T. equiperdum* antigen .

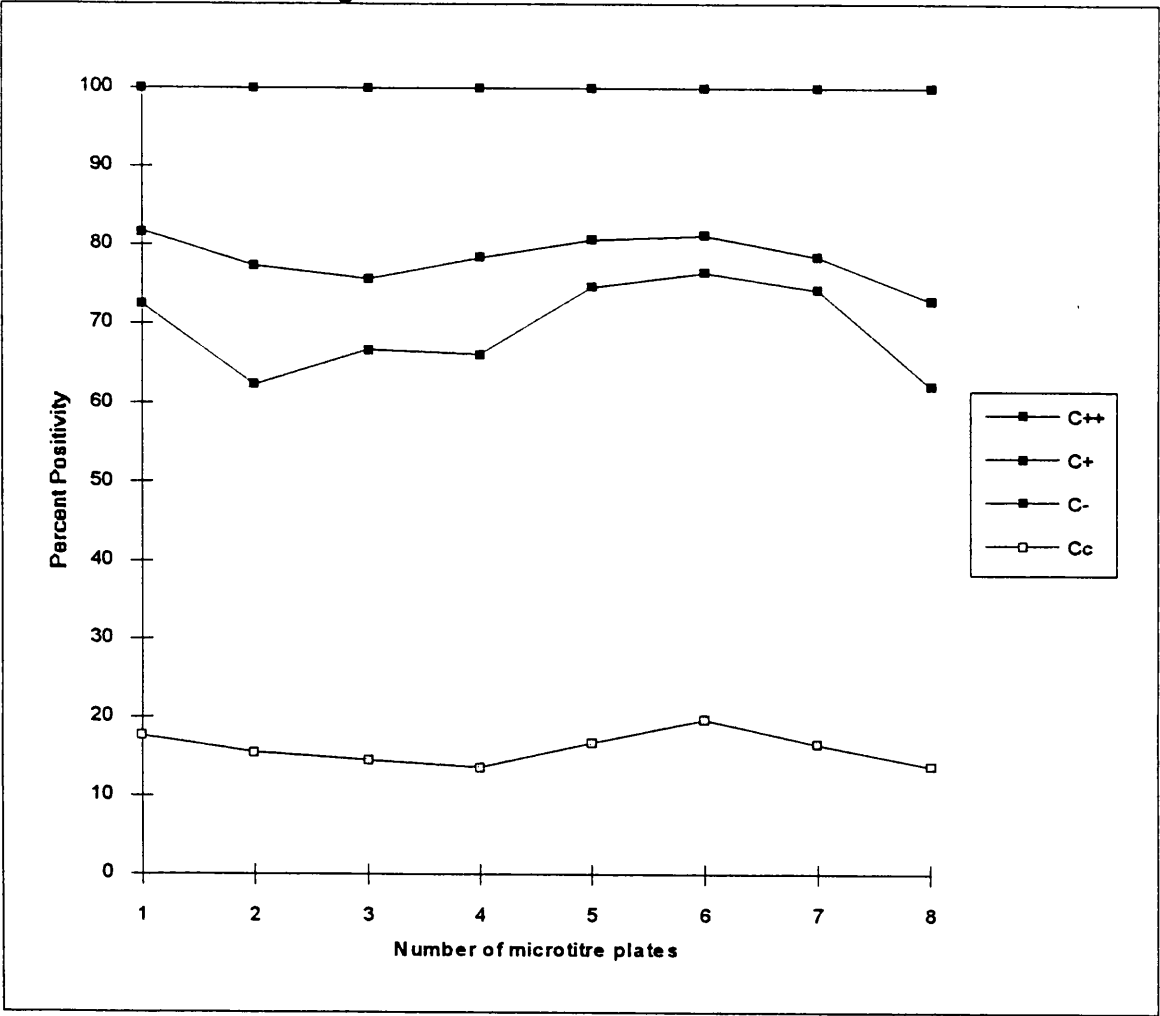
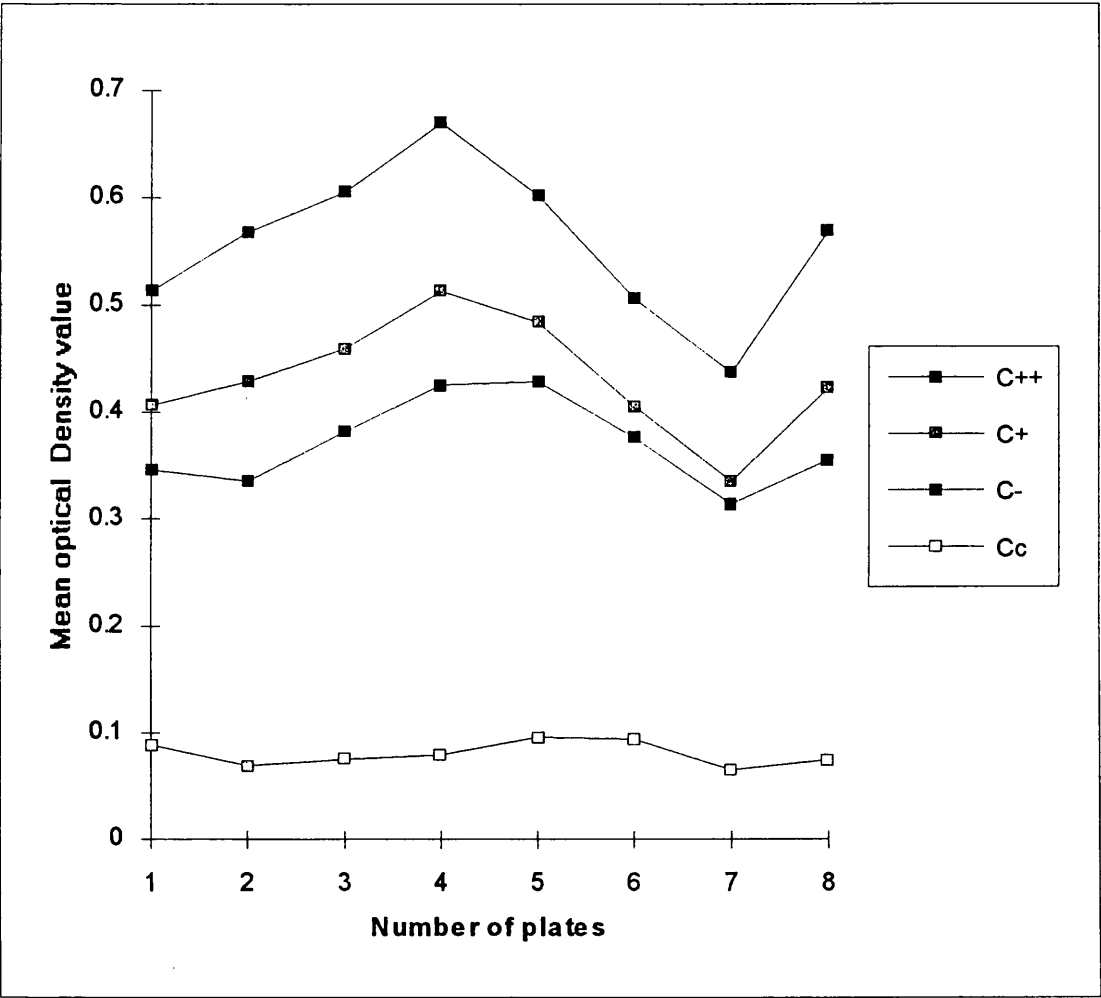


Figure 4.14 Mean Optical Density for the controls in double sandwich ELISA test.





## **Data Expression**

The data expressed in PP values for the C++ control sera used as the 100% positive reference were used to determine the percentage positivity of the test sera on each plate.

## **Cut-off value**

Although there are a number of methods to determine the Seropositive/Seronegative threshold (cut-off ) value, for this study the cut-off value was taken as 2.2 fold the PP value of the negative control (C-) of each plate.

## **IV Laboratory animal inoculation**

Despite the fact that the study areas were selected outside any known tsetse-infested habitat and there was not any documented or suspected cases of *T evansi* it was considered important to inoculate laboratory animals as a supporting method of diagnosis. The laboratory animals used in this study were mice and rabbits.

### **Materials and Methods**

**Mice :** Eighty white mice bought from the Central Disease Investigation Laboratory (the former Pasteur institute) were divided into 10 groups (eight mice per group). Each group were kept in a separate plastic/metal cage, with wood shavings and given an identification number. Each group of 8 mice were sub-grouped into three (for blood inoculation 3 mice; for genital wash inoculation 3 mice; and 2 mice kept as control in each cage) and marked in a separate colour. The mice were fed during the study period with pelleted concentrates and fresh drinking water was provided in plastic bottles.

**Rabbits :** Eight male white rabbits were bought from the above mentioned institute and kept in pairs in plastic cages. They were fed with concentrates and fresh cabbage. Fresh drinking water was also provided.

**Blood and genital washes :** Based on the initial results of the indirect ELISA for the detection of *T. equiperdum* antigen 10 horses of both sex ( 3 from Shirka, 2 from Bekoji, 2 from Assassa, 2 from kofele and 1 from Kore ) were selected for this experiment as donors of blood and genital discharges but only seven horses were made available out of which 5 were females and 2 were males.

Blood and genital discharge were collected from the antigenaemic horses and inoculated by intraperitoneal and intratesticular injection into mice and rabbits.

1) Blood collected from the jugular vein of antigen-positive horses heparinised vacutainer tubes was inoculated into mice (sub-group A) 0.5 ml each intraperitoneally while 0.5ml of genital discharge collected from the same horse was inoculated into mice sub-group B intraperitoneally. The two control mice remained uninfected.

2) Fresh blood and genital washes were taken from three antigenaemic horses and injected into rabbits. Three groups of two rabbits each were subgrouped into Rabbit A and Rabbit B. Rabbit A was injected with 2ml blood while Rabbit B was injected with 2ml of genital wash from the same horse by intratesticular inoculation. The fourth group of two rabbits remained uninfected as control.

Monitoring of mice and rabbits for parasitaemia began 48 hours after inoculation. The mice were bled by snipping the tail and a drop of blood was examined as a wet smear under 40x objective (Olympus CH-2 microscope) using the matching technique described by Herbert and Lumsden in (1976). Similarly the rabbits were also checked by taking blood from the ear vein and examining a wet blood film and buffer coat under 40x objective. The study on the mice terminated after two weeks post inoculation. The rabbits were kept under supervision in the veterinary laboratory at Debre Zeit and blood smears were examined for trypanosomes three times a week until 8 weeks post inoculation.

## Results

### I Clinical Signs

The clinical signs in the 309 horses were divided into four classes. One hundred ninety two did not show any specific disease, 51 horses showed moderate signs of the disease, 56 horses showed signs which involved the genitalia, skin eruptions and poor body condition. Ten horses showed signs which included nervous disturbances. The questionnaires revealed that 35 horses had been treated a number of times previously against dourine although none had been treated in the past month. The distribution amongst the clinical groups was 5 horses in group 1, 11 horses in group 2, 17 horses in group 3 and 2 horses in group 4. There was no clue as to what type of drugs had been administered. However it was known that suramin and quinapyramine used to be in the area but currently these drugs are very scarce.

### II Indirect antibody ELISA

Of the 309 horse sera tested for the detection of anti-*T.equiperdum* antibody 101 samples had percentage positivity values more than 2.2 fold the negative PP value; out of which 77 (76%) were female and 24 (23%) were male. The prevalence in the study areas varied from 12.7% to 34.9% (Table 4.3). The frequency distribution of the test sera showed a clear demarcation of the positive /negative threshold (Figure 4.15). Seventy horses with high percentage positivity for the antibody ELISA also showed moderate to typical clinical symptoms of dourine (Figure 4.16). Three type of analysis have been performed using the two PP's (PP1 and PP2) and and the clinical values.

**Chi-square analysis:** has shown that there is a significant difference in the distribution of animals between the clinical groupings, with prevalence increasing with increasing severity of clinical signs (Table 4.4).

**Figure 4.15** Frequency distribution of horse sera tested for antibody detection.

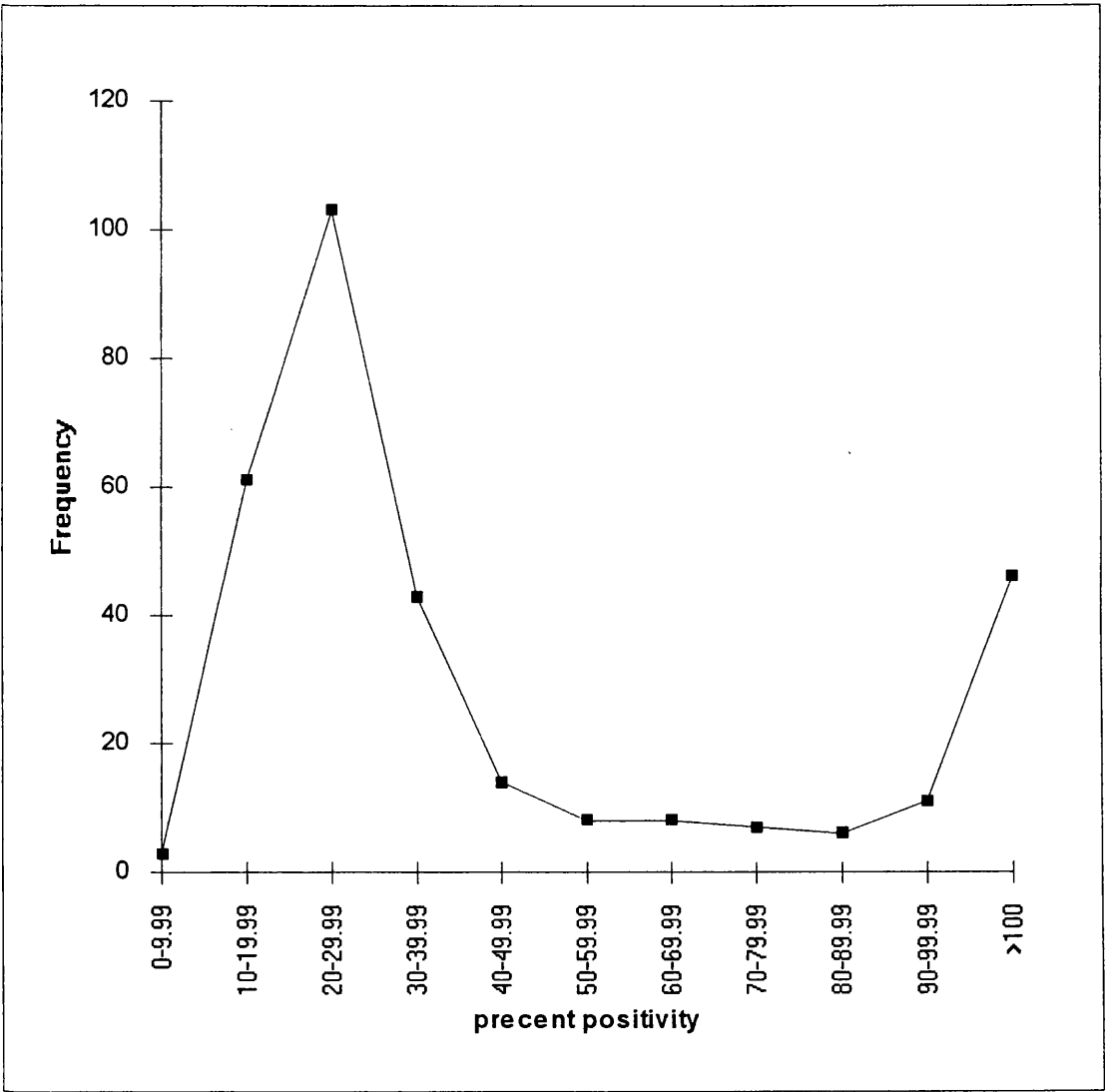
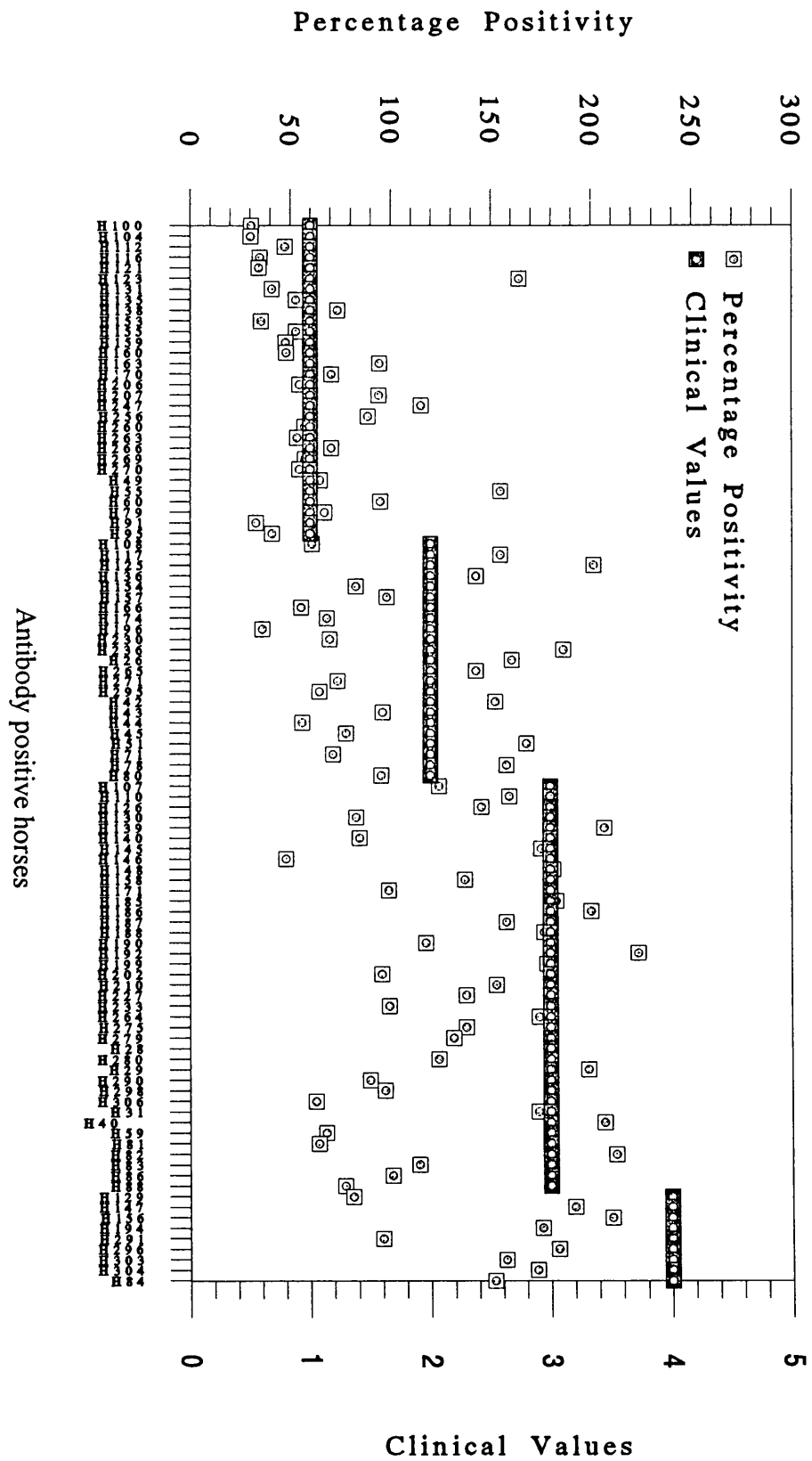


Figure 4.16 Correlation of percent positivity and clinical values in 101 antibody positive horses.



**Table 4.3** The prevalence of antibody positive horses in the study areas

Study area	Number of horses tested	Positive horses	Prevalence
Sagure	47	6	12.7%
Shirka	81	22	27.2%
Bekoji	43	15	34.9%
Dodolla	55	10	18.2%
Assassa	28	4	14.3%
Kofelle	25	7	28%
Kore	30	6	20%

**Table 4.4** Results of Chi-square analysis.

	Clinical		Score	
	1	2	3	4
Positive	31	23	38	9
Negative	161	28	18	1
Prevalence %	16.1	45.1	67.9	90

Chi-sq=73.86, df=3, p<0.001

### III *Trypanosoma equiperdum* antigen detection

Out of the 309 horse sera tested for the detection of circulating *T. equiperdum* antigen 19 horses had percentage positivity values higher than the cut-off value of 2.2 fold the negative control PP values of each plate. However if we consider the frequency distribution pattern over 50 horses were in the upper group i.e had above 80% positivity (Figure 4.17). With regard to the former result ,there were 19 horses, 16 (84. %) mares and 3 (16 %) stallions. The clinical symptoms observed in the field and the antigen level detected were not quite significantly correlated ( $p=0.06$ ) (Figure 4.18). The prevalence of antigen positive sera in the study area ranged from 1.8% to 13.3% (see Table 4.5). Out of these 19 antigen positive sera only eight also showed high percentage positivity on the antibody ELISA. These eight horses showed a variety of clinical signs. One horse showed mild clinical signs of dourine. Six showed typical clinical signs of dourine but limited to the genitalia and skin. One horse showed severe clinical signs including nervous disturbances.

**Chi-square analysis:** In the same clinical groupings the prevalence did not increased with increasing clinical severity (Table 4.6).



**Figure 4.17** Frequency distribution of sera tested using double sandwich ELISA

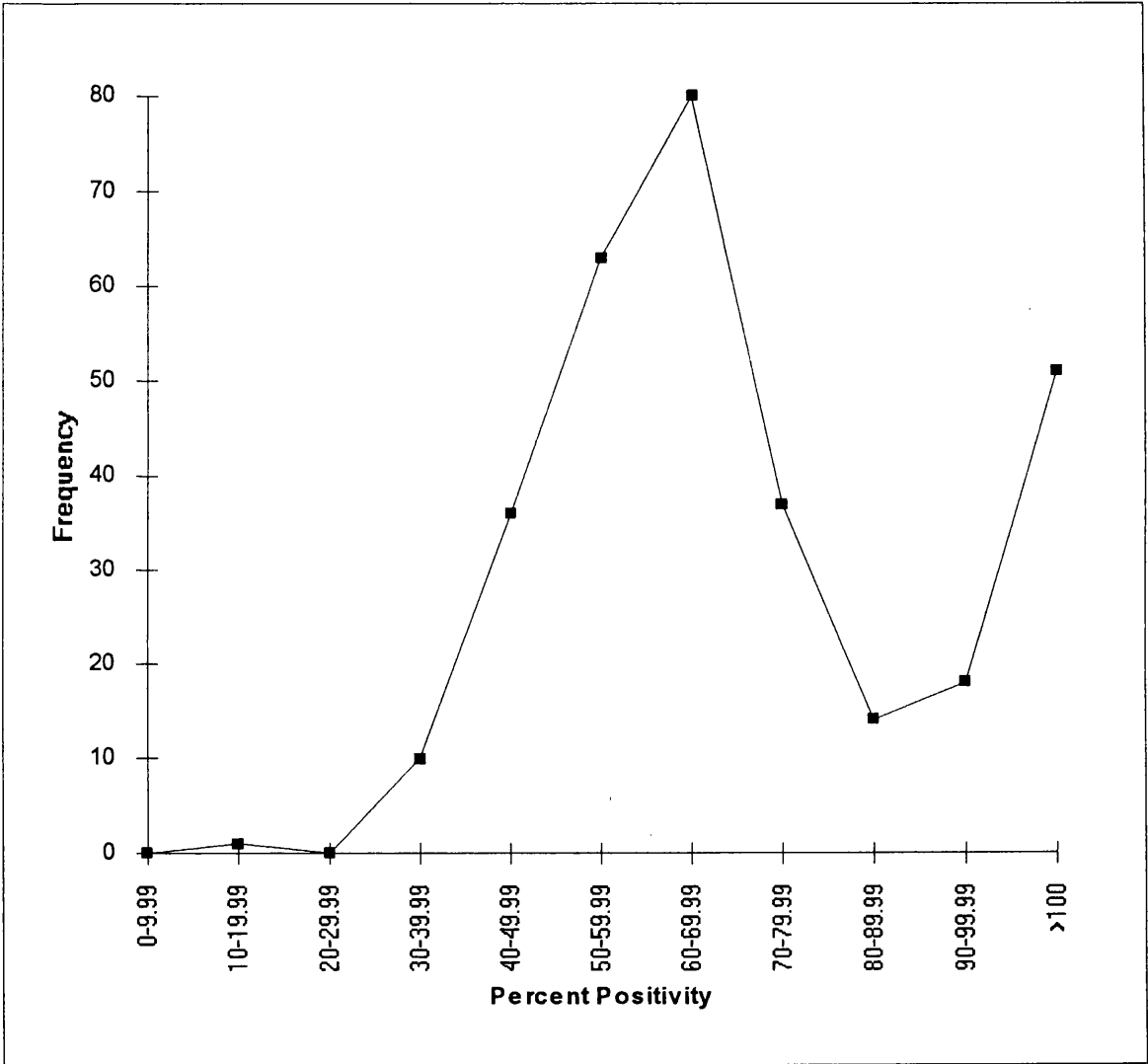


Figure 4.18 Correlation of percent positivity and clinical values in 19 antigen positive horses.

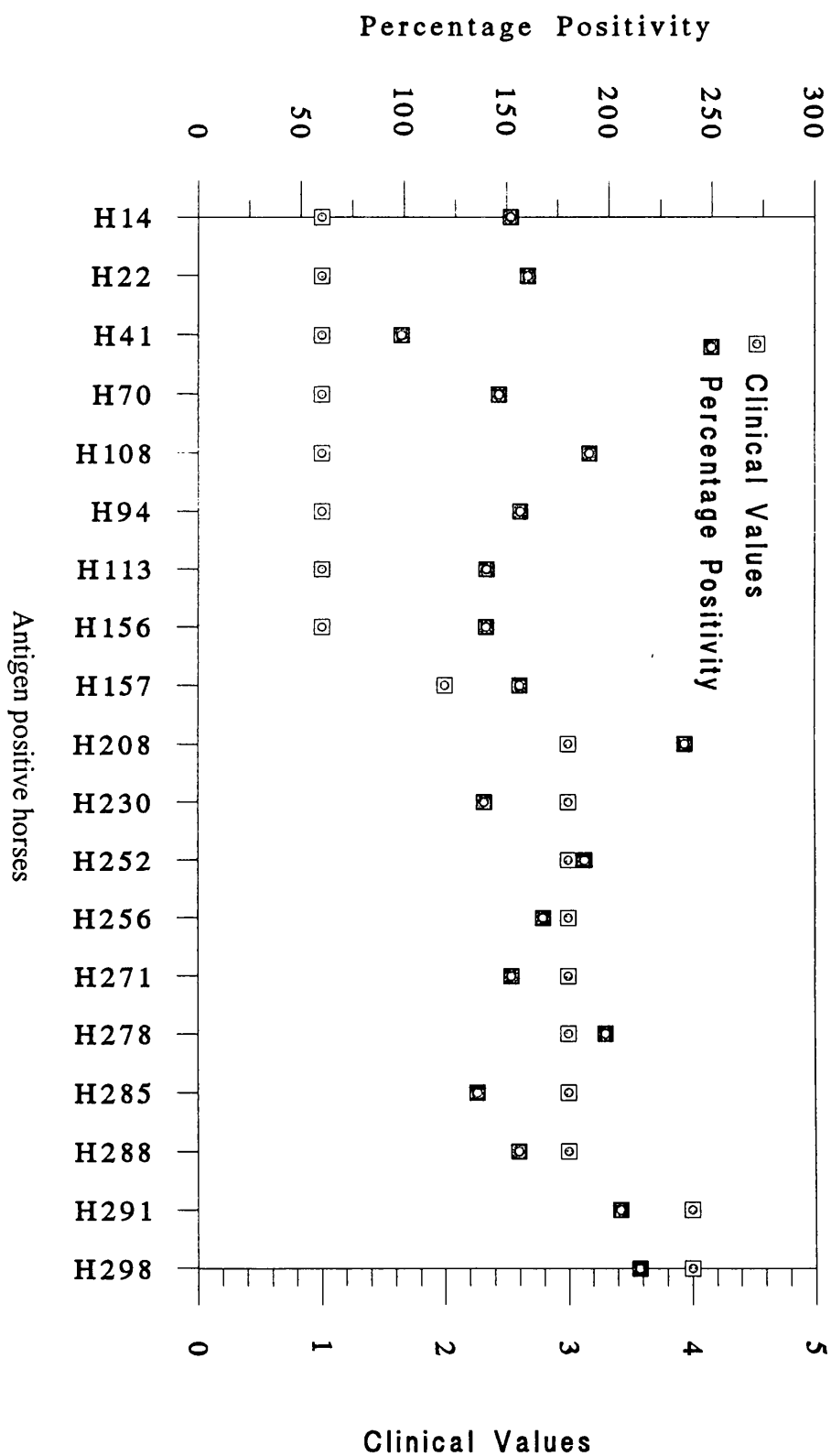


Table 4.5 The distribution of antigen positive horses in the study area

Study area	No. of horses sampled	positive horses	Prevalence
Sagure	47	3	6.4%
Shirka	81	4	4.9%
Bekoji	43	2	4.6%
Dodolla	55	1	1.8%
Assasa	28	2	7.1%
Kofelle	25	3	12%
Kore	30	4	13.3%

**Table 4.6** Results of Chi-square analysis.

	Clinical		Score	
	1	2	3	4
Positive	8	1	3	2
Negative	184	50	48	8
Prevalence %	4.2	2.0	14.3	20

Chi-sq=12.61, df=3,p<0.01

#### IV Laboratory animal inoculation

Efforts made to isolate the parasite in laboratory animals were not successful. Neither the seven groups of mice nor the three groups of rabbits developed a detectable parasitaemia or clinical changes and all remained healthy as did the controls. The experiment terminated for mice 13 days after inoculation but the rabbits were left for another 6 weeks in Debre Zeit Veterinary Laboratory and they were checked three times a week but they remained aparasitaemic and healthy. The follow up was terminated after 8 weeks post inoculation.

#### Discussion

This survey of horses in the Arsi and Bale regions of Ethiopia provides very strong evidence that *T. equiperdum* infections are significant threat to the well-being and productivity of the equine population.

The diagnosis of *T. equiperdum* infections is unfortunately not straightforward and this can lead to difficulties in achieving reliable data on the prevalence and distribution of infections and in the implementation and monitoring of control programmes

The diagnosis of *T. equiperdum* in equines has so far been dependent on clinical observation and the CF test. However, the clinical signs are not pathognomonic and although clinical signs for dourine are useful in making diagnosis, some horses may not show clinical signs (Lingard, 1906; Watson, 1920; Schulz, 1935 and Kasansky as cited by Hoare, 1972). The CF test can give inconclusive results particularly with mule and donkey sera (Williamson and Herr, 1986). In addition the test itself is technically demanding and labour intensive, particularly in terms of the preparation and titration of reagents (Wassall *et al.*, 1991). The application of ELISAs for the detection of *T. equiperdum* infection could improve the efficiency of control measures against this parasite.

Local farmers were aware that the disease had a seasonal character which normally coincided with the breeding seasons. Skin plaques, which are regarded as important symptoms in cases of dourine were not seen during this investigation although, skin eruptions and poor body conditions were prominent. However, Watson (1920) stated that "plaques" are a rare symptom and they can be observed in comparatively few cases. In the early stages of the infection oedema of the genital organs and fever are the rule. As explained by individual farmers in the study areas and observed during the study most of the horses with mild to moderate clinical signs had no feeding problem provided that there was sufficient grazing or feed available. This is in agreement with the experience of Schulz ( 1935 ) in South Africa.

The *T. equiperdum* antigen used in this study for the detection of circulating antibodies performed well and a number of test sera showed high antibody positivity in comparison with the threshold value. Wassall (1991) described the ELISA technique as sensitive and reliable for the diagnosis of *T. equiperdum* and suggested that it should be considered as an internationally approved test for the diagnosis of dourine. Earlier other workers (Caporale *et al.*, 1981; Williamson *et al.*, 1988) have stated that the ELISA has a satisfactory concordance ratio with CFT and can be used to supplement CFT. The antigen used in this study was species specific and this is important in field studies (Luckins and Mehlitz, 1978). The problem of dourine was recognised by farmers in the study area and treatment does take place.

Earlier the indirect ELISA test was shown to be as sensitive as the IFAT in detecting *T. evansi* infections in rabbits (Luckins *et al.*, 1978) and detected trypanosomal antibodies in infected cattle (Luckins, 1977) and camels (Luckins *et al.*, 1979).

Tests for the detection of trypanosomal antigens followed the development of species specific monoclonal antibodies for use in ELISAs (Luckins, 1992). Nantulya and Lindqvist (1989) have detected antigens in cattle between 8 to 14 days after infection with the three tsetse-borne species of pathogenic trypanosomes. Successful treatment of animals is confirmed by the disappearance of antigen from the

circulation (Nantulya, 1990; Nantulya and Lindqvist, 1989). The method has been widely used for *T. brucei* species particularly for the detection of *T. evansi* infection in different species of domestic livestock (Rae and Luckins, 1984; Rae *et al.*, 1989; Payne *et al.*, 1991; Olaho *et al.*, 1993; Pathak *et al.*, 1993; Waitumbi and Nantulya, 1993).

The *T. evansi* monoclonal antibody used in this study was specific for the *T. brucei* subgroup of trypanosomes and it detected circulating antigen in 19 samples of horse sera. This study was conducted outside a tsetse infested area (in the range of 2330 to 2810 meters above sea level), far from any camels and with no record of *T. evansi* either in the study or the surrounding areas,

Attempts to isolate the parasite using laboratory animals (mice and rabbits) were not successful and all the animals inoculated with blood and genital discharge from antigenaemic horses remained aparasitaemic during the study which was terminated 2 and 8 weeks post inoculation respectively. This result is consistent with those of previous studies that *T. equiperdum* is not readily transmitted to laboratory animals from equines (Watson, 1920; Haig, 1948). However Kemenes and Horvath (1986); Ekejindu *et al.*, (1985) and Packhanian (1963) showed that once the parasite is adapted to laboratory rodents it can easily be transmitted by inoculation. Godfrey and Killick-Kendrick (1962) described that the inoculation of small laboratory rodents with blood from animals suspected of being infected with surra is an extremely sensitive method for revealing infections with *T. evansi*. Pegram and Scott (1976) also described it as the best method of detecting *T. evansi* in camels during their investigations in Ethiopia. The failure to detect parasites in the mice and rabbits supported the view that infection with *T. brucei* and *T. evansi* were not present in the horses and that the assay was detecting *T. equiperdum* antigen in the Ethiopian horses.

## Conclusions

This study has enabled the investigator to obtain strong circumstantial evidence that *T. equiperdum*, which is the causative agent of dourine in equines, occurs in the Arsi and Bale regions of Ethiopia. All the three investigation methods (clinical observation, serology tests and laboratory animal inoculation) provided valuable evidence towards this conclusion. In addition all the study sites were in the highlands, and at altitudes outside the known tsetse-infested areas and there was no evidence of *T. evansi* infections in the areas of study. The significant correlation of clinical signs observed in the field with antibody positive and antigen positive horses gives further support for the existence of *T. equiperdum* in the above mentioned study areas. Moreover since equines travel long distances freely for trade and transport purposes in Ethiopia it is believed that the disease may have a much wider distribution than the study areas and especially in places where there are large equine populations.

## Recommendations

Although the control of dourine ideally requires strong regulations related to the movement of equines in the endemic areas, and the eradication of positive animals these measures may not be easy to implement in Ethiopia because neither the government nor the peasants are ready to accept these requirements. For these reasons the control methods recommended for the control of *T. equiperdum* in Ethiopia at the present time are as follows:

- 1) Treatment of infected equines. *Trypanosoma equiperdum* is sensitive to those trypanocidal drugs which are in use for the control of *T. evansi* and *T. brucei* infections. For these reasons it is recommended that curative treatments should be used against dourine on the basis of clinical signs particularly in areas where the presence of the disease is recognised (Arsi and Bale) based on the results of this study. However, it is important to establish the efficacy of these drugs in treating



*T.equiperdum* infections in Ethiopian horses. The sandwich antigen-detection ELISA could be used for this purpose.

2) Although it may not be easy to convince the farmers the other possible control strategy is to separate breeding and working equines. All the males which are not required for breeding should be castrated at an early age. Those selected for breeding should be strictly supervised to avoid contact with strange horses. If available, artificial insemination could be helpful particularly for expensive horses to reduce the risk of infection.

3) From the results achieved in this study it is recommended that indirect ELISAs for the detection of antibody and antigen could be used to extend our investigations of the prevalence, incidence and distribution of *T. equiperdum* infections to all areas of Ethiopia which have large equine populations.

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